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BELGIAN SOCIETY FOR MICROBIOLOGY

National Committee for Microbiology
of

The Royal Academies of Science
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Annual Symposium

Microbes in the spotlight

Academy Palace, Brussels

October 19th 2018

BSM 2018 Annual Symposium

Microbes in the spotlight

Brussels, October 19, 2018

08.30	*Registration – Poster mounting*
09.00	*Welcome address:* GR Cornelis, Chairman of BSM
09.10	***Session D: Host and microbial interactions*** *(Chair: L Gillet)* Plenary speaker: **Xavier Nassif, Université Paris Descartes & Institut Necker Enfants Malades, FR** *How bacterial microvasculature interaction modifies the clinical presentation of septicemia, the paradigm of meningococcemia*
09.55	***Short talks – Session D:*** Lionel Schiavolin, IBENS, France: *Shigella hijacks Ago2 activity to promote the rupture of its vacuole* Hannah Frost, ULB: *A novel interaction between C4BP and the Enn protein of Group A Streptococcus*
10.15	***Session C: Medical and Veterinary Microbiology*** *(Chair: P Cos)* Plenary speaker: **Hilde Revets, UAntwerpen, BE** *Why do we need new polio vaccines for polio eradication?*
11.00	***Short talks – Session C:*** Jolien Vitse, Ghent University: *The effect of ciprofloxacin on Stenotrophomonas maltophilia: communication or competition?* E.R. Job, VIB Ghent : *Broadened immunity against influenza by vaccination with computationally designed Influenza virus N1 neuraminidase constructs*
11.20	*Coffee break and poster viewing*

11.40	***Session B: Applied and Environmental Microbiology*** *(Chairs: I George - N Boon)* Plenary speaker: Prof. Victor de Lorenzo, CSIC, Madrid, SP *How environmental bacteria conquest new chemical landscapes*
12.25	*Short talks – Session B:* Laurens Maertens, SCK/CEN: *Understanding copper and silver based antimicrobials and their potential in space applications* Cristina García-Timermans, Ghent University: *Single-cell bacterial characterization using flow cytometry and Raman spectroscopy*
12.45	*General assembly*
13.00	*Lunch and poster sessions*
14.30	***Session A : General Microbiology*** *(Chair: G. Cornelis)* Jeff Errington, Newcastle University, UK *Title: L-form bacteria: penicillin, lysozyme and recurrent infection*
15.20	***Short talks – Session A:*** Sander Govers, KULeuven: Bacterial protein aggregates can serve as epigenetic memory of previous torments Frédéric Goormaghtigh, ULB: Single-cell imaging and characterization of *Escherichia coli* type II persister cells to ofloxacin
15.40	***BSM honorary Lecture*** *(Chair: X De Bolle)* Christine Jacobs-Wagner, Microbial Sciences Institute, Yale University, USA *What is special about the Lyme disease pathogen*
16.30	Bergey medal to Niall Logan (Glasgow University, UK) Announcement of poster and short communication awards
17.00	End of the meeting

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Abstracts Posters Section D; Host and Microbe interaction

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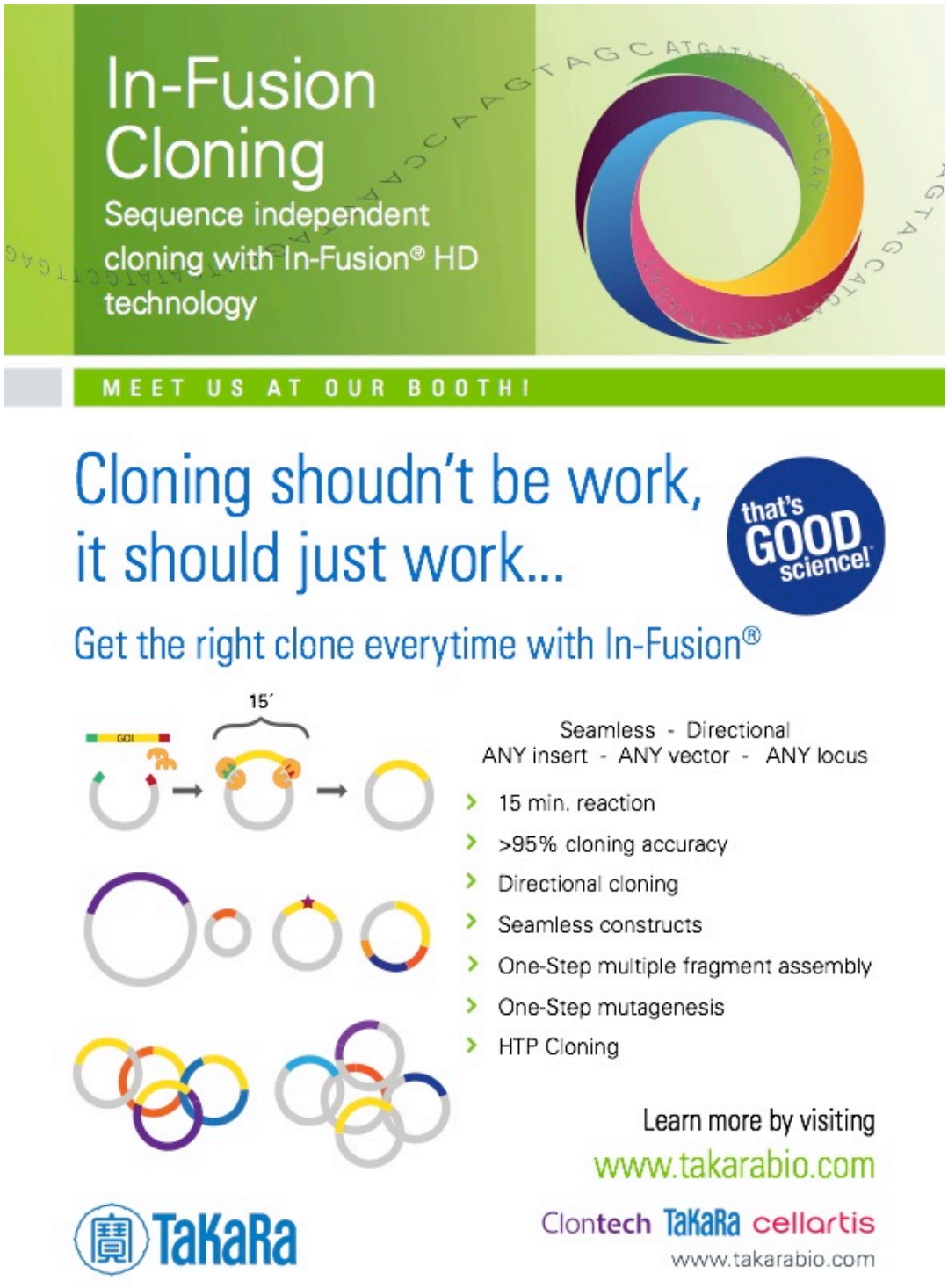
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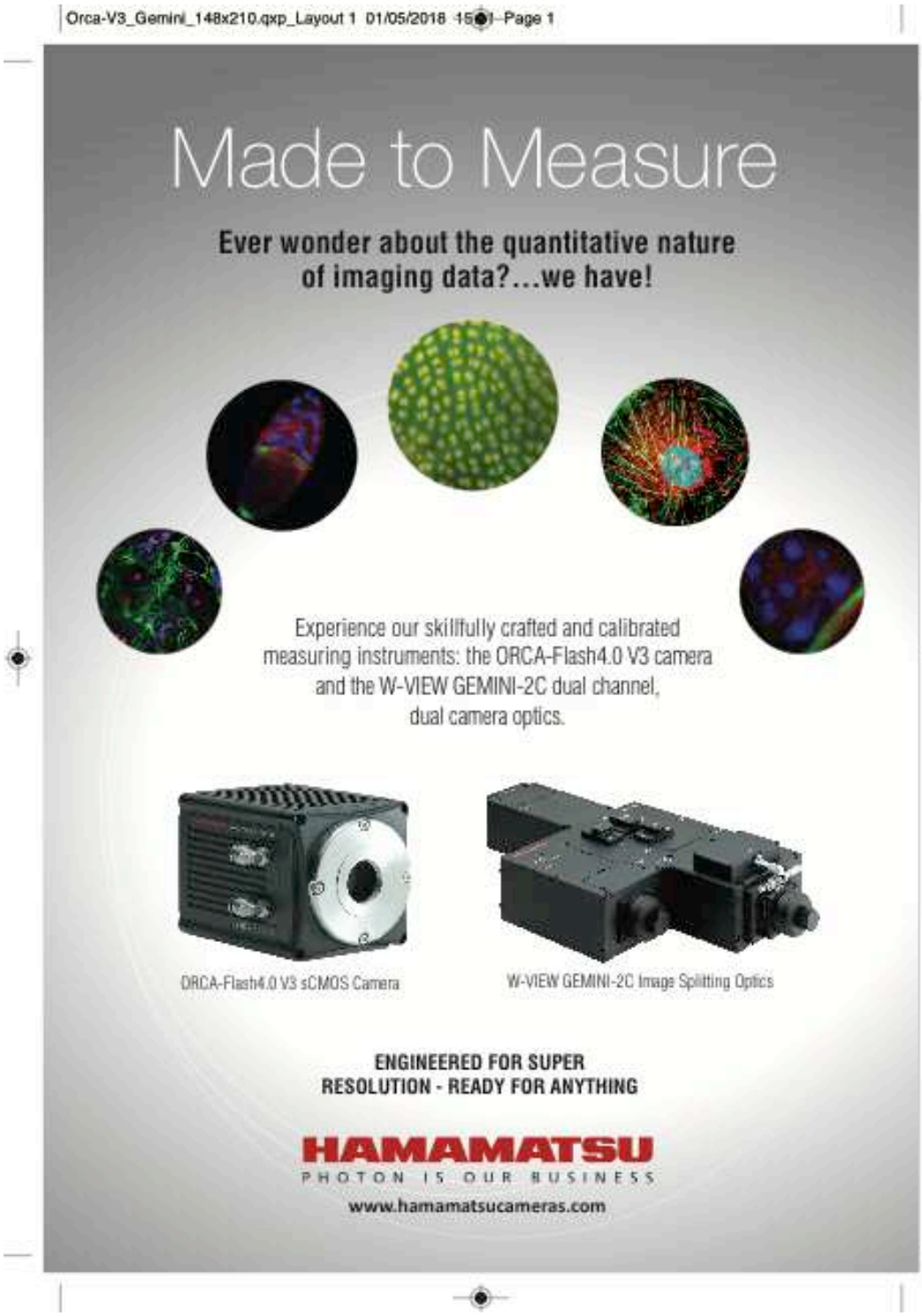

ABSTRACTS INVITED LECTURES

PLENARY LECTURES

SECTION D

HOST AND MICROBE INTERACTIONS

How bacterial microvasculature interaction modifies the clinical presentation of septicemia, the paradigm of meningococcemia

Xavier Nassif

Institut Necker-Enfants Malades, Paris

Neisseria meningitidis is an extra cellular bacterial pathogen responsible for a sepsis which is a paradigm of bacterial septicemia. Once in the bloodstream, this bacterium has the ability to give meningitis after crossing the blood brain barrier and/or to be responsible for a very severe sepsis with a thrombotic/leakage syndrome which can lead to a deadly purpura fulminans. The major pathogenic feature of *N.meningitidis* is its ability to tightly interact with the brain and peripheral microvessels and to form microcolonies on the apical surface of the endothelium. Pathogen interaction with microvessels is mediated by bacterial type IV pili and two receptors on endothelial cells, CD147 and the β2 adrenergic receptor. The former facilitates bacterial adhesion on endothelial cells, whereas the latter is responsible for bacterial signaling. In this presentation the consequences of this interaction and how it relates to the clinical presentation and the outcome of the infection will be addressed.

SECTION C

MEDICAL AND VETERINARY MICROBIOLOGY

Why do we need new polio vaccines for polio eradication?

Hilde Revets, Ilse De Coster and Pierre Van Damme

Hilde Revets

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Polio was once a disease feared worldwide, striking suddenly and paralysing mainly children for life. The Global Polio Eradication Initiative, the largest private-public partnership for health has reduced polio by 99%. Though the current oral vaccine has been effective in eliminating polio in many geographies, it can cause vaccine-related polio itself in extremely rare occasions and be responsible for circulation of mutant strains. With global eradication of wild-type polio within sight, but an ongoing risk of outbreaks from vaccine derived viruses (VDPV) one of the final steps of the eradication strategy is to prepare stockpiles of safer novel monovalent poliovirus vaccines for use in outbreak control.

We describe the recently created novel purpose-built contained facility, Poliopolis, to study the immunogenicity, shedding and genetic stability of two novel serotype 2 oral poliovirus vaccine candidates in healthy adult volunteers. To safeguard any potential risk for release into the environment of the vaccine strains, several techniques for elimination or inactivation of the GMOs at any time during and at the end of the study were put in place. The design and construction of Poliopolis and the rationale for the different procedures put in place, practical challenges for implementation and results will be discussed.

SECTION B

APPLIED AND ENVIRONMENTAL MICROBIOLOGY

How environmental bacteria conquest new chemical landscapes

Víctor de Lorenzo.

Systems Biology Program, National Center of Biotechnology-CSIC, Darwin 3, Cantoblanco-Madrid, Spain. vdlorenzo@cnb.csic.es.

Bacteria that colonize sites polluted by industrial waste are capable of metabolizing synthetic and recalcitrant chemicals that have been in the biosphere for only a few years. Such an *evolution-in-action* scenario provide an optimal experimental to inspect in real time how metabolic networks expand to reach out new chemical structures. The capability to degrade novel molecules is orchestrated by the integration of environmental and physiological signals into regulatory systems that tightly control the expression of genes that are in charge of catabolizing such molecules. In order to understand how bacteria solve such a multi-objective optimization challenge we have examined the still-evolving 2,4-dinitrotoluene biodegradative pathway in *Burkholderia* sp. DNT. The *dnt* pathway of this bacterium apparently evolved from a precursor naphthalene degradation route and the first enzyme (2,4-dinitrotoluene dioxygenase) maintains some activity towards its earlier substrate. Examination of both *in vivo* reactions and the associated regulatory system suggests that ROS production is the first bottleneck that evolving pathways have to overcome for dealing with novel compounds and that oxidative stress caused by faulty metabolism of the substrates becomes a major source of genetic diversification. When the same *dnt* route is passed to other bacterial hosts, consequences do vary. In the case of *Pseudomonas putida*, the mutagenic effects of ROS are quenched by the powerful reductive metabolism of this bacterium. In contrast, expression of the *dnt* pathway in *E. coli* results in a genetic diversification regime that is mediated by the *rpoS*-ruled general stress response. Evolutionary and ecological consequences of this state of affairs—and some hints for engineering new biocatalysts—will be discussed.

SECTION A

GENERAL MICROBIOLOGY

L-form bacteria: penicillin, lysozyme and recurrent infection

Yoshikazu Kawai, Katarzyna Mickiewicz, Ling Juan Wu and Jeff Errington

Jeff Errington

Centre for Bacterial Cell Biology, Newcastle University, NE2 4AX, UK

The peptidoglycan cell wall is a defining structure of the bacteria. It is the target for our best antibiotics and fragments of the wall trigger powerful innate immune responses against infection. The genes for peptidoglycan synthesis are present in most bacterial lineages, suggesting that the wall emerged early in cellular evolution. Surprisingly, many bacteria can switch, sometimes spontaneously, into a cell wall deficient "L-form" state in which they become completely resistant to many cell wall active antibiotics. Remarkably, L-form growth is completely independent of the complex FtsZ-based division machine that is essential in almost all bacteria. Proliferation occurs, instead, by a seemingly haphazard process involving membrane blebbing or tubulation and scission, leading to progeny of irregular size and shape. The switch to this mode of proliferation seems to require only the upregulation of membrane synthesis, leading to an increased surface area to volume ratio. L-forms may provide insights into how primitive cells proliferated before the evolution of the cell wall. Recent results have highlighted remarkable antagonistic interactions between lysozyme and β-lactams with important potential implications for antibiotic evasion and recurrent infection. Finally, we detected L-form like cells in the urine of most patients participating in a recent longitudinal study of elderly patients with recurrent UTI, and show that the isolated bacteria can readily switch in and out of the L-form state.

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Mercier et al., 2016, *Nature Microbiology* 1, 16091.
Kawai et al., 2018, *Cell* 172, 1038–1049.

BSM HONORARY LECTURE

What is special about the Lyme disease pathogen?

Christine Jacobs-Wagner

Microbial Sciences Institute, Yale University, USA

To replicate successfully, cells must perform numerous tasks, which are stochastic in nature. Yet, cellular replication is incredibly robust, with each division producing daughter cells that are competent for self-replication. In my talk, I will briefly discuss the importance of intracellular organization for successful bacterial multiplication. Then, I will focus the discussion on how differences in processes underlying bacterial growth may contribute to pathogenesis and disease.

ABSTRACTS SHORT TALKS

SECTION B: APPLIED AND ENVIRONMENTAL MICROBIOLOGY

Single-cell bacterial characterization using flow cytometry and Raman spectroscopy

Cristina García-Timermans[a*], Peter Rubbens[a,b], Frederiek-Maarten Kerckhof[a], Ruben Props[a], - Willem Waegeman[b], Nico Boon[a]

[a]CMET, Center for Microbial Technology and Ecology, Ghent University, Gent, Belgium;[b]KERMIT, Department of Data Analysis and Mathematical Modelling, Ghent University, Gent, Belgium

Single-cell techniques, such as flow cytometry and Raman spectroscopy, allow to characterize bacterial populations and communities. As these tools become more widely used, there is an increasing need to understand their advantages and limitations. For instance, flow cytometry is much more high-throughput than Raman, but it measures about ten times less parameters. How does this difference translate into practice and when should each approach be used?

In this work, we compared the capacity of flow cytometry and Raman spectroscopy to identify within a bacterial culture their subpopulations. After harvesting *E. coli* at different growth stages, we measured them using flow cytometry and Raman spectroscopy. While flow cytometry did not give enough information to distinguish single-cell variations, it could detect the differences amongst the groups. Raman spectroscopy could identify single-cell variations, and the spectral information could help identify the cell components that made these cells functionally different. Thus, we propose to consider flow cytometry as a bulk phenotyping technique that can detect shifts in microbial populations, and Raman spectroscopy to identify adequately single-cell variations.

Understanding copper and silver based antimicrobials and their potential in space applications

Laurens Maertens

Research institute: SCK-CEN, EHS, Interdisciplinary Biosciences, Microbiology Unit, Boeretang 200, 2400 Mol, SCK•CEN mentors: Dr. Ir. Rob Van Houdt, Dr. Ir. Pieter Monsieurs

University: UNamur, Research Unit in Biology of Microorganisms: Rue de Bruxelles 61, 5000 Namur, University promoter: Prof. Jean-Yves Matroule

Despite rigorous cleaning procedures and the presence of antimicrobial silver, bacteria such as *Cupriavidus metallidurans* can survive in the drinking water supplies aboard the International Space Station (ISS). Many studies indicate that bacterial behaviour is altered during space flight with an important role for the Hfq protein, which is required for the regulatory function of many sRNA-mRNA (small regulatory RNA - messenger RNA) interactions. However, very little is known about the existence and function of sRNAs in *C. metallidurans*, or their regulatory impact on transcriptional networks in this strain. We hope to achieve an overview of sRNA regulation in *C. metallidurans*, with a focus on mechanisms for metal resistance and proliferation in nutrient-limiting conditions.

In a first approach, sRNAs were identified from a pre-existing RNA-Seq dataset of ^{238}U-exposed *C. metallidurans* NA4 cells. Extracted RNA had been ribosomally depleted, and sequenced on an Illumina MiSeq® platform using paired-end reads of 125 nt. Reads were aligned to the NA4 reference genome, and resulting strand-specific bam files were converted to wig format. This database was fed to the ANNOgesic pipeline for small regulatory RNA detection, using standard parameters for fragmented reads, and filtering based on stability of secondary structure.

1301 putative sRNA genes were found to be expressed in all three biological replicates of both the ^{238}U-exposed cells and the controls. 2905 putative sRNA genes were found to be located antisense to CDSs (coding sequences), while 1376 putative sRNA genes were found in intergenic regions. sRNA expression reached levels as high as CDS expression in both ^{238}U-exposed cells and controls. In the control condition, 35 putative sRNA genes and 1268 CDSs had an average RPKM value (Reads Per Kilobase Million) above 20. In the ^{238}U-exposed cells, only 18 putative sRNA genes and 1319 CDSs had an average RPKM value above 20. Putative sRNA genes were often differentially expressed in the ^{238}U-exposed condition, in comparison to the control condition. Of the 100 sRNA genes with the highest average expression over all replicates and conditions, 29 had p-values smaller than 0.05 when a two-tailed unpaired Student t-test was performed.
Even though many putative sRNA genes were detected, some at expression levels similar to highly expressed CDSs, and some showing differential expression between both conditions, many improvements to sRNA calling can still be made. False positives can be filtered out using prediction of (new) transcription start sites, defining an experimental 'noise' coverage level, and thorough manual curation. New experimental setups will also be more tailored towards sRNA detection, employing shorter, single reads, and including short RNAs that would usually be rejected in both sample preparation and bioinformatic analysis.

ABSTRACTS SHORT TALKS

SECTION C: MEDICAL AND VETERINARY MICROBIOLOGY

Broadened immunity against influenza by vaccination with computationally designed Influenza virus N1 neuraminidase constructs

E.R. Job[1,2], T. Ysenbaert[1,2], A. Smet[1,2], I. Christopoulou[1,2], T. Strugnell[3], E.O. Oloo[3], R.P. Oomen[3], H. Kleanthous[3], T. U. Vogel[3], X. Saelens[1,2]

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[2] *Department of Biomedical Molecular Biology, Ghent University, 9052 Ghent, Belgium*

[3] *Sanofi Pasteur, Research North America, Cambridge, Massachusetts, USA*

Split inactivated influenza vaccines remain one of the primary preventative strategies against severe influenza disease in the population. However, current vaccines are only effective against a limited number of matched strains. The need for broadly protective vaccines is acute due to the high mutational rate of influenza viruses and multiple strain variants in circulation at any one time. The neuraminidase (NA) glycoprotein expressed on the influenza virion surface, has recently regained recognition as a valuable vaccine candidate. We sought to broaden the protection provided by NA within the N1 subtype by computationally engineering consensus NA sequences. Three NA antigens (NA5200, NA7900, NA9100) were designed based on sequence clusters encompassing three major groupings of NA sequence space; (i) H1N1 2009 pandemic and Swine H1N1, (ii) historical seasonal H1N1 and (iii) H1N1 viruses ranging from 1933 till current times. Recombinant NA proteins were produced as a vaccine and used in a mouse challenge model. The design of the protein dictated the protection provided against the challenge strains. NA5200 protected against H1N1 pdm09, a Swine isolate from 1998 and NIBRG-14 (H5N1). NA7900 protected against all seasonal H1N1 viruses tested and NA9100 showed the broadest range of protection covering all N1 viruses tested. By passive transfer studies and serological assays, the protection provided by the cluster-based consensus (CBC) designs correlated to antibodies capable of mediating NA inhibition. Importantly, sera raised to the consensus NAs displayed a broader pattern of reactivity and protection than naturally occurring NAs potentially supporting a predictive approach to antigen design.

The effect of ciprofloxacin on *Stenotrophomonas maltophilia*: communication or competition?

Jolien Vitse, Simon Devos, Wouter Van Putte, Bart Devreese

Laboratory for Microbiology – Protein Research Unit, Department of Biochemistry and Microbiology, Ghent University
K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

Cystic fibrosis (CF) is currently the most common, lethal genetic disorder among the Caucasian population, with an incidence of 1 in 2000 - 3000 births in the European Union (World Health Organization). CF is caused by mutations in both copies of the 'cystic fibrosis conductance regulator' (*CFTR*) gene, that lead to a defective CFTR ion channel. An important complication associated with CFTR mutation is the accumulation of a thick viscous mucous layer in the airways. For this reason, patients with CF are very susceptible to chronic airway infections which drastically reduce the life expectancy(Lipuma, 2010). Important bacterial species associated with CF are: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Stenotrophomonas maltophilia* and *Burkholderia cenocepacia*. CF-associated species frequently show higher resistance against many currently used antibiotics, partly due to the formation of polymicrobial biofilm communities.

Inter- and intra-species cell-cell interactions and communication are key aspects for species survival within these communities. The secretion of virulence factors, signaling molecules (quorum sensors) and membrane vesicles (MVs) enables bacteria to interact with the host as well as other bacterial cells(Depluverez et al., 2016). In this context, the secretion of bacterial MVs has gained much attention lately. We demonstrated that the fluoroquinolone antibiotic ciprofloxacin induces MV production in *S. maltophilia* (Devos et al., 2017). However, ciprofloxacin leads to the production of three distinct populations of MVs: the classic outer membrane vesicles (OMVs), larger vesicles containing both inner and outer membrane (inner-outer membrane vesicles (IOMVs)), and IOMVs that are enriched with fimbriae. The observation of fimbriae on these MVs could be a key feature for biofilm formation in *S. maltophilia* or even in other species. Therefore, the major fimbrial subunit (SMF-1) encoded by the *SMLT0706* gene was deleted in *S. maltophilia* to gain more insight into the function of the MV-associated fimbriae. On the other hand, the ciprofloxacin-induced MVs appeared to be highly toxic for *P. aeruginosa.* Moreover, previous electron microscopic research indicated the presence of phages or phage-tail like structures in these MV isolates (Devos et al., 2017). Liu et al. (2013) identified a phage-tail like bacteriocin (maltocin) secreted by *S. maltophilia* P28, containing an endolysin, which has antibacterial activity against several Gram-Negative and Gram-Positive species, including *P. aeruginosa*. In order to obtain more insight into the origin of the toxic effect of the ciprofloxacin-induced MV isolates on *P. aeruginosa*, a knock-out mutant of the *SMLT1054* gene encoding a phage-related endolysin will be generated as well.

The fluoroquinolone antibiotic ciprofloxacin can evoke a process of communication on the one hand and competition on the other hand in *S. maltophilia*. However, the exact mechanism and the importance of the MVs in this process needs to be further elucidated.

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ABSTRACTS SHORT TALKS

SECTION D: HOST AND MICROBE INTERACTIONS

Shigella hijacks Ago2 activity to promote the rupture of its vacuole

***Lionel Schiavolin*[1], *Didier Filopon*[1], *Mariette Bonnet*[2], *Brice Sperandio*[3], *Philippe Sansonetti*[3,4], *Guy Tran Van Nhieu*[2] & *Lionel Navarro*[1]**

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MicroRNAs (miRNAs) are critical fine-tuners of host immune responses in both plants and animals. Each small RNA associates with an Argonaute (Ago) protein which is the central component of a multiprotein RNA-induced silencing complex (RISC). The small RNA further directs AGO-RISC onto sequence complementary mRNA targets to trigger their post-transcriptional silencing. This is manifested by endonucleolytic cleavage (slicing), and/or translational inhibition of the mRNA targets. The functional relevance of individual miRNAs in controlling bacterial infection just begins to be elucidated. However, there is no evidence implicating the human miRNA machinery in this process, as previously reported in plants. Here, we have used *Shigella* and HeLa cells as an *in vitro* infection model system to study the role and regulation of Ago2 in host-bacterial interactions. During the course of *Shigella* infection, the bacterium induces its host cell internalization in a macropinocytic-like process by means of its type III secretion system. It further rapidly escapes from its phagocytic vacuole to reach the cytosol, a step which is critical for *Shigella* intracellular lifestyle and that requires the bacterial type III effector IpgD. Using live microscopy, we found that Ago2 is transiently recruited at *Shigella* entry foci and that Ago2 knocked-down or knocked-out cells exhibit a delay in *Shigella*-induced vacuole rupture. A similar delay in vacuole rupture was observed in cells depleted of other miRNA factors, supporting a role for the miRNA pathway in this process. Using a complementation assay in *ago2*$^{-/-}$ cells, we further show that the miRNA-mediated translational inhibition activity of Ago2 is essential for rapid *Shigella*-induced vacuole rupture, while its slicing activity is dispensable for this process. Additionally, we show that the phosphorylation at Ser387 of Ago2 (Ago2 S387P) is required for vacuole rupture. This phosphorylation is dependent on the Akt survival pathway, which is known to be targeted by IpgD. Finally, we provide genetic evidence suggesting that IpgD has evolved to trigger Ago2 S387P to promote vacuole rupture. Altogether, this study demonstrates for the first time a critical role of Ago2, and of the human miRNA pathway, in host-bacterial interactions. This work also provides novel insights into the process of *Shigella*-induced vacuole rupture, a phenomenon that remains poorly characterized at the mechanistic level.

A novel interaction between C4BP and the Enn protein of Group A streptococcus

Hannah Frost (1), Martina Sanderson-Smith (2), David De Oliveira (2), Ailish Cleary (2), Mark Davies (3), Mark Walker (4), Andrew Steer (5), Anne Botteaux* (1) and Pierre Smeesters* (1)

**Contributed equally*

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3. Department of Microbiology and Immunology, University of Melbourne, Australia

4. Australian Infectious Diseases Research Centre, University of Queensland, Australia

5. Group A Streptococcus Research Group, MCRI, Melbourne, Australia

Background: Group A *Streptococcus* (GAS) M proteins have an exceptional ability to induce phagocytosis resistance and aid in establishing infections. Binding of the potent inhibitor of complement, C4BP, to M proteins has been characterized to a structural level. Binding of C4BP to whole GAS has been demonstrated for 89 of 100 *emm*-types. However, studies using purified proteins found no interaction with M proteins from 13 of 22 *emm*-types positive for whole cell binding, suggesting other C4BP binding partners exist.

Methods and Results: C4BP-binding motifs were defined based on sequence patterns in C4BP-binding M proteins. We discovered predicted binding sites in several GAS Enn proteins. The gene for Enn is present in 90% of over 1400 GAS genomes and up to two thirds contain predicted C4BP-binding motifs. 9 C4BP-binding GAS strains with M proteins negative for C4BP binding were selected. The ability of the whole bacteria to bind C4BP was confirmed using surface plasmon resonance and flow cytometry. Enn proteins from these C4BP-binding GAS strains with or without the predicted C4BP-binding motif were produced. Binding of C4BP from human serum was observed by pull-down assays for 7 Enn proteins and was negative for the 2 other Enn proteins. We have mapped binding to the N-terminus of Enn for one of the C4BP binding proteins and determined essential residues required for C4BP binding using targeted mutagenesis.

Conclusions: This work suggests that Enn proteins may also play a significant role in binding of C4BP at the GAS surface. The impact of this interaction on virulence and vaccination requires further investigation.

ABSTRACTS SHORT TALKS

SECTION A: GENERAL MICROBIOLOGY

Bacterial protein aggregates can serve as epigenetic memory of previous torments

Sander Govers1, Julien Mortier1, Antoine Adam2, Abram Aertsen1*

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Protein misfolding and aggregation are typically perceived as inevitable and detrimental processes tied to a stress- or age-associated decline in cellular proteostasis. A careful reassessment of this paradigm in the *Escherichia coli* model bacterium revealed that the emergence of intracellular protein aggregates (PAs) was not related to cellular aging but closely linked to sublethal proteotoxic stresses such as exposure to heat, peroxide, and the antibiotic streptomycin. After removal of the proteotoxic stress and resumption of cellular proliferation, the polarly deposited PA was subjected to limited disaggregation and therefore became asymmetrically inherited for a large number of generations. Many generations after the original PA-inducing stress, the cells inheriting this ancestral PA displayed a significantly increased heat resistance compared to their isogenic, PA-free siblings. This PA-mediated inheritance of heat resistance could be reproduced with a conditionally expressed, intracellular PA consisting of an inert, aggregation-prone mutant protein, validating the role of PAs in increasing resistance and indicating that the resistance-conferring mechanism does not depend on the origin of the PA. Moreover, PAs were found to confer robustness to other proteotoxic stresses, as imposed by reactive oxygen species or streptomycin exposure, suggesting a broad protective effect. Our findings therefore reveal the potential of intracellular PAs to serve as long-term epigenetically inheritable and functional memory elements, physically referring to a previous cellular insult that occurred many generations ago and meanwhile improving robustness to a subsequent proteotoxic stress.[1]

[1] Govers *et al.* (2018). PLoS Biol., 16, e2003853.

Single-cell imaging and characterization of Escherichia coli type II persister cells to ofloxacin

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Bacterial persistence to antibiotics refers to the capacity of small subpopulations within clonal populations to tolerate antibiotic treatments. Two types of persisters were defined, depending whether they are carried over from stationary phase (type I) or generated stochastically during steady-state growth (type II). Molecular mechanisms involved in type II persistence are still unclear. Here we have set up an experimental framework allowing single-cell imaging and characterization of type II persister cells during an entire persistence cycle i.e. before, during and after the antibiotic treatment. This methodology allowed for the first time the observation of type II persister cells present in a population of wild-type cells treated with ofloxacin. Our data reveal that type II persister cells are actively growing before the ofloxacin treatment and experiencing DNA damage as judged by the induction of the SOS response. At the time of recovery, upon antibiotic removal, type II persister cells undergo a second SOS induction accompanied by SOS-independent cell filamentation. With time, SOS induction decreases and cell division resume at multiple locations within the filament. We speculate that upon ofloxacin removal, DNA replication resumes and the DNA replication machinery encounters topoisomerase-DNA complexes, leading to the second SOS induction. DNA is subsequently repaired by homologous recombination and nucleoids segregate along the filament, allowing cell division to resume and give rise to viable progeny. The checkpoint mechanism underlying SOS-independent filamentation remains unknown.

ABSTRACTS POSTERS

SECTION A: GENERAL MICROBIOLOGY

Molecular dissection of the peptide specificity of the competence activator ComR in streptococci

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Competence development generally allows a bacterial subpopulation to transiently acquire exogenous DNA, which is important for genome plasticity, DNA repair and nutrition. In addition, competence development in streptococci is generally associated with the production of antimicrobial compounds (e.g. bacteriocins) resulting in lysis of surrounding bacteria and extracellular release of DNA and polysaccharides. The complete process requires an important energy investment to the recipient cells. Consequently, it is a tightly regulated process. In the salivarius streptococci group, the activation of competence relies on the cell-to-cell regulatory system ComRS, where ComR is a transcriptional regulator of the RRNPP superfamily which displays an N-terminal DNA-binding domain (HTH) and a C-terminal TPR-domain which interacts with the pheromone ComS or XIP. The low level of conservation of TPR-domains and the diversity of peptides sequences, together with the strictness *vs*. promiscuity of diverse ComR orthologues in presence of non-cognate peptides, suggest a co-evolution between TPR-domains and their corresponding pheromones under species-specific specialization.

The ComRS-DNA ternary complex crystal structure of *Streptococcus thermophilus*, combined with a mutational analysis, allowed to propose a novel activation mechanism, which was unknown for this family of regulators. This mechanisms is based on HTH-domain sequestration in absence of XIP. Moreover, key information about peptide-TPR domain binding and recognition was disclosed, highlighting regions likely to be involved in XIP specificity. In order to verify their implication in the selection of the pheromone, we have tested competence activation by chimeric ComRs (*i.e.* hybrid proteins between ComR orthologs of *S.thermophilus* and *S.vestibularis*). Strict ComR of *S. vestibularis* was chosen due to the role of this species as opportunistic human pathogen.

The importance of variations in the ComRS cross-talk capacity for streptococci population dynamics are currently studied in order to evaluate if the ComRS permissiveness has important consequences for interspecies communication and competition.

In brief, this project will contribute to a better understanding of the physiological function of the signaling system ComRS in competence and predation in streptococci. Comprehending the molecular basis of the ComR-XIP signaling and cross-talk between streptococci will open avenues for the development/selection of antimicrobial strategies for the control of pathogenic streptococci.

Multiple proteins arising from a single gene: role of the Spa33 variants in the *Shigella flexneri* T3SS regulation

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Shigella flexneri, a gram-negative bacterium is the causative agent of the shigellosis or bacillary dysentery in humans. Shigellosis is an invasive disease of the colonic epithelium caused by a severe inflammatory reaction and subsequent mucosal destruction. The invasion and dissemination in epithelial cells of *Shigella* are mainly dependent of a type 3 secretion system (T3SS) which mediates the translocation of virulence proteins into host cells. T3SSs are composed of three major parts: an extracellular portion (the needle), a basal body and a cytoplasmic bulb (C-ring). After cell contact, proteins (called translocators) are secreted to form a pore (translocation pore) in the host cell membrane. This pore serves as a gate for secreted virulence proteins (called effectors) to gain access to the host cell cytoplasm. The mechanism underlying T3SS activation by host cell contact is still misunderstood but implicates the transmission of a signal from the tip of the needle to the base, resulting in the secretion of cytoplasmic protein (MxiC), which serves as an internal plug before cell contact. Spa33 (33-kDa) has been identified as an essential C-ring component of *Shigella* T3SS since the *spa33* mutant (Δ*spa33*) is unable to form a needle and to secrete any proteins. To further understand the role of Spa33 in T3SS, we have first cloned the *spa33* gene in an expressing vector (pET30a), fused to a 6-histidines tag at both ends and observed that five fragments were detectable by immune-detection. Of these, three fragments were well expressed; one of 33-kDa corresponding to the Spa33 full-length (FL), one of around 12-kDa which was previously identified by McDowell et al. and called $Spa33^{C}$, a smaller one of around 8-kDa (called here fragment X). Two other fragments were less expressed; one of around 15 kDa (fragment Y) and one around 11 kDa (fragment Z). By a mutational approach, we have identified fragment X as a product of an internal start codon in the *spa33* gene. We have shown that this fragment is required for secretion of T3SS substrates. Fragment Y was identified as a slippage product from the *spa33* gene, but its absence does not impact T3SS. In addition, we have found that several single amino acid substitutions in Spa33 deregulate secretion of virulence proteins, suggesting that Spa33 is implicated in the T3SS regulation. Finally, we have shown that Spa33 interact with both MxiC and MxiI strengthening our model in which Spa33, MxiC and MxiI act together to control the T3SS after host cell contact.

Unveiling the specialized metabolites of *Streptomyces lunaelactis* : Particular interest in the unusual *fev* cluster

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Moonmilk deposits of limestone caves host a rich microbiome, among which antibiotic producing-Actinobacteria represent one of the most abundant phyla, making these carbonate deposits an appealing unexplored biotope for bioprospecting producers of novel molecules with therapeutic properties. *Streptomyces lunaelactis* is the first moonmilk-dwelling Actinobacteria that has been fully characterized and genome mining revealed that this species possess 37 biosynthetic gene clusters (BGCs) involved in the production of specialized (secondary) metabolites. The aim of our work is to unveil the metabolome of *S. lunaelactis* with the hope of finding novel natural compounds.

This poster illustrates the implication of one particular cluster (identified by antiSMASH analyses) in the biosynthesis of specialized metabolites produced by the strain *S. lunaelactis* identified by mass spectrometry.

The conserved synteny and high identity between *fev* and *bag* clusters, together with the identification of bagremycin A in the extracts of *S. lunaelactis* MM109T strongly suggests that synthesis of ferroverdins and bagremycins is mediated by the same BGC. Taking account of all these data combine with the previous publications on the *fev* and *bag* clusters, we proposed a hypothetical biosynthetic pathway where the *fev* cluster of *S. lunaelactis* produce these two different specialized metabolites. These results show that the genome mining is not only useful to evaluate the ability to produce specialized metabolites of a strain but also allow the prediction of biosynthetic pathways.

Phages that farm: infection dynamics imposed by the phage carrier state unveil a new phage bet-hedging strategy

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Bacteria are engaged in a never-ending struggle with their viral predators, bacteriophages. As these viruses mediate lysis or lysogenic conversion of the bacterial cell, phage – host infection dynamics play a central role in our modern life by affecting industrial processes, human health, and earth's ecology. Fully unraveling and modelling these dynamics, as well as the mechanisms governing them, is indispensable for understanding, predicting and future steering of microbial ecosystems. Long term phage-host co-existence has resulted in the intricate co-evolution of virus and host. This triggers the development of sophisticated interactions of which restriction-modification systems and CRISPR-Cas are merely two examples. Another recently discovered interaction is the formation of a phage carrier state, in which a delay of the integration event of a temperate phage in its host chromosome results in the emergence of a phage free subpopulation that is transiently resistant to superinfection. This strategy allows these farming phages to foster a reservoir of bacterial cells that afterwards can be "harvested". We investigated the infection dynamics of P22-*Salmonella* Typhimurium, at population level in bioreactors as well as the underlying molecular mechanisms using time-lapse microscopy. This reveals that the phage carrier state empowers the phage to invest in vertical and horizontal transmission at the same time by simultaneously forming lysogens and phage free siblings that are later employed for the production of virions.

Comparison of the properties of *Pseudomonas aeruginosa* biofilm cells dispersed with Vapor Nano Bubbles to cells derived from other dispersal methods

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Biofilm infections are challenging to treat due to enhanced antibiotic tolerance and biofilm dispersal is considered as one of the strategies that could be used to increase antibiotic efficacy on biofilms. We recently developed a novel strategy to disrupt biofilms, using laser-generated Vapor Nano Bubbles (VNBs). Previous work showed that VNB disrupted biofilms show increased antibiotic susceptibility compared to undisrupted biofilms. In the present study we compare antibiotic tolerance and the transcriptome of *P. aeruginosa* cells released from a biofilm via VNB-treatment, to the tolerance and the transcriptome of cells released with other dispersal methods.

P. aeruginosa PAO1 biofilms were grown in flow-cells at room-temperature for 4 days. Prior to VNB biofilm disruption, gold nanoparticles were added to biofilms. Subsequently, biofilms were exposed to a green (561 nm) pulsing laser. This creates a nano-sized water-vapor bubble around the gold nanoparticle, which disrupts the biofilm. Biofilm dispersal was also induced with 500 µM sodium nitroprusside (SNP) or by a sudden increase of the carbon source concentration (18 mM glutamate). The cells released from the biofilm were collected and their susceptibility to tobramycin (10 µg/ml) and colistin (16 µg/ml) was determined (using time-kill assays). As a control we used cells that were released spontaneously (i.e. without external trigger) and planktonic cells. In addition, RNA was collected from these cells, gene expression profiles were determined using Illumina-based RNA sequencing.

Dispersed cells (irrespective of the dispersal method) showed reduced killing compared to planktonic cells, when exposed to tobramycin or colistin. While complete eradication (7 log) was obtained for the planktonic culture after 5h incubation with tobramycin, only a 4 log decrease was observed for dispersed cells. Similarly, the planktonic culture (7 log) was completely eradicated after 2 hours treatment with colistin, whereas only a 3 log decrease was obtained for dispersed cells.

When comparing spontaneously dispersed cells to cells dispersed by an external trigger, 55 genes showed differential expression. These genes are involved in anaerobic respiration (e.g. *nir*-genes) and in pyochelin production (pch-genes).

342 genes were found to be differentially expressed in VNB treated cells in comparison to spontaneously dispersed cells and cells dispersed by SNP or glutamate. This includes genes encoding proteins involved in antibiotic resistance (e.g. *mexY, mexPQ*), virulence (e.g. *pvdG, pscOP*) and pillus production (e.g. *xphA*).

Dispersed cells (irrespective of the dispersal method) have an enhanced tolerance to tobramycin and colistin.

RNA sequencing revealed that gene expression in VNB-dispersed cells is different from that in cells dispersed with other approaches.

Replication fork targeting by the Tn*3*-family transposon Tn*4430*: a genetic and biochemical study

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Transposition reactions generally generate DNA intermediates that need to be processed or repaired by the host cell. However, the mechanisms whereby transpososomes recruit or communicate with the host DNA-processing machineries are poorly understood. Here, we address this issue using the replicative transposition mechanism of the Tn*3*-family transposon Tn*4430* from *Bacillus thuringiensis* as a model system. Recent *in vivo* and *in vitro* studies on this element converge on a new "replisome hijacking" model according to which the transposon integrates into replication forks as a direct mechanism to recruit the host replication machinery for its own duplication.

The aim of this project is to validate this model by developing complementary genetic, cytological and biochemical approaches. Functional interaction between the transposition and replication machineries is examined *in vivo* by establishing the integration profile of Tn*4430* in different genetic backgrounds where replication fork progression is affected. Supporting the model of "replisome hijacking", the results show that the insertion of the transposon preferentially occurs in regions where the progression of the replication fork is altered, such as the replication terminus of the chromosome or DNA regions that contain repeats of short palindromic sequences known to interfere with replication by forming secondary DNA structures.

This unique targeting mechanism of Tn4430 is supported by biochemical data showing that the purified transposase TnpA specifically binds to replication fork-like structures in vitro, and that it can use it as an efficient substrate for the strand transfer reaction. Future work aims at looking at physical interactions between TnpA and the replication machinery in life cells and in vitro.

Characterization of a bacterial-like TetR transcription factor involved in fatty acid metabolism in the archaeal model organism Sulfolobus acidocaldarius

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One of the most distinctive characteristics of archaea are their membrane lipids, which contain isoprenoids linked by an ether bond to a glycerol moiety instead of the ester-linked fatty acids that are present in bacteria and eukaryotes. Despite this absence of fatty acids in the lipids, many archaeal genomes contain genes that are predicted to be involved in fatty acid metabolism. *Sulfolobus acidocaldarius*, a hyperthermoacidophilic crenarchaeon, contains a cluster of genes encoding enzymes involved in fatty acid and lipid metabolism. Growth tests, both using plate assays and liquid cultures in presence of fatty acids of different chain lengths (C_4-C_{18}), demonstrate that *S. acidocaldarius* can utilize fatty acids as a carbon and energy source. The extensive gene cluster also harbors a gene encoding a TetR-family regulator named SaFadR. Gene expression analysis by RNA-seq and qRT-PCR comparing a SaFadR deletion strain with the wild type strain showed that the entire gene cluster is repressed by the regulator. In presence of long-chain acyl-CoA molecules, the ability of SaFadR to bind to its targets is disrupted *in vitro*, probably leading to derepression of the targets. Additionally, characterization of the binding sites based on footprinting experiments and co-crystal structures of FadR-DNA complexes have provided insights into the molecular interactions that underly the regulatory mechanism. These results indicate that this gene cluster is involved in fatty acid degradation.

Methyl arachidonyl fluorophosphonate inhibits *Mycobacterium tuberculosis* thioesterase TesA and mycobacterial biofilm formation

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Mycobacterium tuberculosis, the causative agent of human tuberculosis, harbors a thick cell wall with a high lipid content. Phthiocerol dimycocerosates (PDIM) and phenolic glycolipids (PGL), considered as major virulence elements, are involved in mycobacteria cell wall permeability and drug resistance. The biosynthesis of PDIM and PGL involve multiple enzymes, including thioesterase A (TesA, a type II thioesterase). Here, we expressed and purified the recombinant *Mycobacterium tuberculosis* TesA protein and assessed its enzymatic activity using various acyl-CoA substrates. Among the several screened inhibitors, methyl arachidonyl fluorophosphonate (MAFP) and tetrahydrolipstatin (THL) were found to inhibit TesA activity. MAFP covalently modified the active site serine in the predicted TesA catalytic triad, inhibited biofilm development and increased vancomycin susceptibility of *Mycobacterium bovis* BCG. These results warrant the development of TesA inhibitors as valuable anti-tuberculous drug candidates.

Conformational changes of the catalytic domain of Rel protein of *Thermus thermophilus*

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(p)ppGpp is a universal alarmone that reprograms bacterial metabolism and triggers its stress-survival mechanisms. In *Escherichia coli,* (p)ppGpp is mainly synthesised by RelA and hydrolysed by SpoT. In most other bacteria, there is just one enzyme called Rel that catalyses both the hydrolysis and synthesis reactions. The bifunctional proteins must switch between these opposing activities without simultaneous activation of both.

The current work aims to shed light on the molecular mechanisms and regulation of the production and degradation of (p)ppGpp by the bifunctional Rel proteins. We have determined the structures of the catalytic domain of Rel from *Thermus thermophilus* (ttRelcd) in complex with different nucleotides. The structures reveal that the binding of certain nucleotides triggers a notable conformational change in the domain carrying out the reaction. One state of the protein allows for the synthesis reaction and prevents the hydrolysis of ppGpp, and in the other, opposingly, the hydrolysis centre is accessible for ppGpp and the synthesis reaction is prevented. The dynamic changes of the domains in the cycle of ppGpp synthesis and hydrolysis were confirmed with smFRET measurements. Moreover, smFRET allows to track the changes of the protein in a very detailed manner, and shows which nucleotide triggers a conformational change and thus initiates a specific reaction. Our results indicate that the preferred state of ttRelcd is the one of active hydrolysis, whereas GDP is required to trigger the switch to the ppGpp-synthesis state. These different states of the enzyme clearly prevent the simultaneous activation of the opposing activities of ttRel protein. Together, this knowledge has given us a deeper insight into the regulation of ppGpp production, which is a crucial step in understanding the regulation of bacterial stress response.

The major component of the Type III translocase self-assembles into the exported protein receptor

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Type III secretion (T3S), a protein export pathway common to Gram-negative pathogens, like enteropathogenic *E. coli* (EPEC), comprises a trans-envelope syringe, the injectisome, with a cytoplasm-facing translocase channel. The translocase comprises an internal EscR, S, T heteropentameric ring that forms the channel proper and is decorated by the EscU dimer, and enveloped by the major EscV nonamer. EscV has a large cytoplasmic domain, thought to act as a receptor for T3S secretory substrates and forms a 5nm ante-chamber leading to the membrane channel. To understand the mechanism of assembly and function of the T3S translocase we reconstituted membrane assembly *in vivo* of EscV alone or in co-complex with EscRSTU in a non-EPEC lab strain and *in vitro* using inverted inner membrane vesicles. In parallel, we identified similar EscV-containing injectisome-derived sub-complexes from EPEC. EscV forms stable nonameric self-assemblies independent of other T3S components and dynamic co-complexes with EscRSTU alone or within the injectisome. Nonameric EscV is necessary and sufficient to act as a receptor for exported effectors. Live cell imaging revealed that EscV assembles at the cell periphery, in several membrane-associated multi-component clusters. These findings advance molecular understanding of injectisome assembly and pave the way for the structural and functional characterization of the membrane-associated translocation reaction.

NlpC/P60 endopeptidases LytA and LytB are essential peptidoglycan hydrolases of the cell cycle of *Lactobacillus plantarum*

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The cell wall is a fundamental structure for the survival and the integrity of bacteria, as it establish cell shape, integrity and protection from internal osmotic pressure. In gram-positive bacteria, peptidoglycan (PG) is the most abundant compound of the cell wall. The peptidoglycan hydrolases (PGHs) are enzymes able to degrade PG, playing a major role in several processes as PG turnover and stability, daughter cell separation, autolysis and host-bacteria recognition. In *L. plantarum* WCFS1, twelve PGHs candidates have been found by genome sequencing and comparison with well-characterized PGHs, including four NlpC/P60 endopeptidases.

In this work we explore the function of the four endopeptidases of *L. plantarum* (LytA, LytB, LytC, LytD), focusing the attention on the functional relationship between LytA and LytB and the interplay between endopeptidases and cell cycle proteins. Firstly, we reported that LytC$^-$ and deletional Δ*lytD* mutants do not have any apparent impact on cell morphology. Secondly, we showed that Δ*lytA* mutants tend to form aggregates; the same result is obtained by LytA depletion by promoter substitution strategies showed that cells. Moreover, LytA inactivation can be associated with the presence of elongation defects and defects in FtsZ localization. Since the Deletion phenotype of LytA, MreB1 and PBP2b are similar, we advanced the hypothesis that LytA could be implicated in the elongation process and in the orientation of the Z-ring. The *in-vivo* study of Δ*lytB* mutants gives a similar inactivation phenotype by comparison with FtsEX, a linker complex between cytoskeleton and PGHs, meaning that they could both being regulators of the division frequency and position. Lastly, we showed that Δ*lytB* together with LytA depletion causes elongation failure and anarchic division.

In conclusion, LytA and LytB seems to have different and complementary roles for the functionality of the cell cycle. We also proposed a model that can explain their global activity. Future works will investigate on the identification of LytA and LytB interactants and the confirmation of the role of LytC and LytD in PG recycling.

Protein-protein interactions in bacterial persistence

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Previous research in our group has demonstrated a central role for the conserved GTPase Obg in mediating persistence. Unraveling the precise functioning of Obg-mediated persistence will shed light on this transient phenomenon and identify possible targets for anti-persister therapies. To identify novel direct interaction partners of Obg, an innovative photo-crosslinking technique was used. This method allows covalent binding of direct binding partners *in vivo*, which is an important feature concerning the transient nature of persistence. The unnatural photo-reactive amino acid *p*-benzoyl-L-phenylalanine (*p*Bpa) is incorporated at specific locations of the Obg protein. Under influence of UV radiation, the carbonyl oxygen of *p*Bpa crosslinks to any carbon-hydrogen bond of molecules within a radius of 3 angstrom. We used this method to incorporate *p*Bpa in Obg from *Escherichia coli* (ObgE). By experimental optimization, we were able to efficiently incorporate *p*Bpa at different residues located on the surface of ObgE and $ObgE_{D246G}$ (mutant form deprived of the persister function). Crosslinking of ObgE with possible interaction partners was detected and the interacting molecules were identified using high resolution liquid chromatography-mass spectrometry. Results are currently being confirmed and verified using a bacterial-two hybrid approach and pull-down assays. Further testing of the identified direct interaction partners will result in a selection of genes specifically involved in the Obg-mediated persistence pathway. The full understanding of this pathway will contribute to the development of anti-persister therapies. We hypothesize that targeting Obg-mediated persistence will significantly reduce the number of persister cells in bacterial populations, thereby facilitating clearing of infections by conventional antibiotics.

Verstraeten N. Knapen WK, Kint CI, Liebens V, Van den Bergh B, Dewachter L, Michiels JE, FuQ, David CC, Fierro AC, Marchal K, Beirlant KJ, Versées W, Hofkens J, Jansen M, Fauvart M, Michiels J. 2015. Obg and membrane depolarization are part of a microbial bet-hedging strategy that leads to antibiotic tolerance. Mol Cell: 59:9-21

Accelerating Comparative Genomics with Short Oligonucleotide-based Genomic Signatures

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Oligonucleotides, also called k-mers, are the foundation for many genomics tools, ranging from assembly to comparative genomics, to genome indexing, as well as many metagenomics tools. However, these tools operate primarily using longer oligonucleotides with length typically above 15. Here we explore the potential of using oligonucleotides with a length shorter than 10, for which most combinations are present in any bacterial genome. By analyzing the occurrence frequency of all possible oligonucleotides of a given length, we obtain a genomic signature which is relatively well conserved within bacterial genomes. Using a signature based on tetranucleotides it is possible to distinguish different bacterial species from each other. Similarly, it is possible to differentiate distantly related strains from closely related strain of the same species using hexanucleotides. Both methods are extremely fast compared to alignment-based methods. When the number of combinations is relatively small, signatures can also be used to visualize bacterial genomes, which can help direct further analysis. We are currently working on creating a tool which integrates all the above in a user-friendly manner. In order to assess our results, we used two databases. The first database contained 134 genomes from the Belgian Coordinated Collection of Microorganisms, which had a reliable taxonomy. The second dataset consisted of over 112000 genomes retrieved from the RefSeq database, for which the taxonomy is more prone to errors. With both datasets, the separation between genomes belonging to different species and genomes belonging to the same species was very clear. Closely related strains could also be distinguished from distantly related strains in most cases.

Biosynthesis and function of the nickel-pincer cofactor of lactate racemase

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The nickel-pincer nucleotide (NPN) (Fig. 1) is a new cofactor that we identified in lactate racemase (LarA). In LarA, NPN is tethered to a lysine and forms a tridentate pincer complex that coordinates nickel through one carbon and two sulfur atoms. Although similar complexes have been previously synthesized, there was no prior evidence for the existence of pincer cofactors in enzymes.

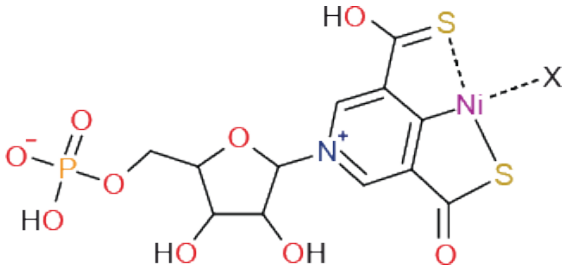

Figure. 1. The nickel-pincer nucleotide cofactor (NPN).

This cofactor is synthesized from nicotinic acid dinucleotide (NaAD) by three biosynthetic enzymes, i.e. LarB, LarC, and LarE. We showed that LarB carboxylates the pyridinium ring of NaAD and cleaves the phosphoanhydride bond to release AMP. The resulting biscarboxylic acid intermediate is transformed into a bisthiocarboxylic acid species by two single-turnover reactions in which sacrificial desulfurization of LarE converts its conserved Cys176 into dehydroalanine. Finally, nickel-containing LarC catalyzes the CTP-dependent nickel insertion into the bisthiocarboxylic acid species, generating the NPN cofactor. This reaction, known in organometallic chemistry as a cyclometalation, is characterized by the formation of new metal-carbon and metal-sulfur σ bonds. LarC is therefore the first cyclometallase identified in nature. We characterized LarC reaction, purified the NPN cofactor, and determined the structure of the C-terminal part of LarC.

A bioinformatics study showed that LarA homolog genes and genes coding for the biosynthetic enzymes are found in 10 % of all studied prokaryotic genomes and even in some eukaryotes, showing the large distribution of LarA homologs. However, it appears that the majority of these LarA homologs are not lactate racemases, bot other kinds of isomerases. We already identified two new reactions catalyzed by LarA homologs: malate racemization and gluconate-mannonate epimerization, yet many other isomerization reactions are still unknown.

The bioinformatics study further identified that genes coding for the biosynthetic enzymes are present without *larA* homolog in an additional 15 % of the studied genomes. This suggests that other unknown NPN-dependent enzymes, catalyzing other types of reactions, are present in these species.

EPIGENETIC REGULATION OF *BURKHOLDERIA CENOCEPACIA* J2315 AND K56-2 PHENOTYPES BY DNA METHYLTRANSFERASES

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Respiratory tract infections by the opportunistic pathogen *Burkholderia cenocepacia*, member of the *Burkholderia cepacia* complex (Bcc), often lead to severe lung deterioration in cystic fibrosis (CF) patients. New insights in how to tackle these infections might emerge from the field of epigenetics. The most-studied form of epigenetic regulation of gene expression is DNA methylation. Methyltransferase enzymes (MTases) interact with characteristic recognition sites on the DNA strand and transfer a CH_3-group from a methyl donor, mostly S-adenosyl methionine (SAM), to an adenine or cytosine base. As methylated bases are less accessible for DNA binding proteins, methylation at specific regulatory regions allows bacteria to regulate gene expression at the level of transcription.

Although DNA methylation regulates gene expression in several bacteria, the exact role and function of specific DNA MTases in *B. cenocepacia* remains unclear. In the present study, *in silico* predicted DNA MTase genes BCAL3494 and BCAM0992, conserved in strains J2315 and K56-2, were deleted, and phenotypic characteristics of the resulting deletion mutants ΔBCAL3494 and ΔBCAM0992 were determined.

Overall, planktonic growth was not affected (i.e. no differences in population density after 24 or 48h, and no difference in growth rate between wild type and mutant strains). However, unlike wild type strains, planktonic cultures of mutant strains ΔBCAL3494_J2315 and ΔBCAL3494_K56-2 formed a biofilm-like pellicle structure at the air-liquid interface under static conditions. In addition to this, planktonic cultures of these mutants contained substantially larger cell clusters (as observed using flow cytometry and fluorescence microscopy after LIVE/DEAD staining) compared to wild type. The other mutant strains, ΔBCAM0992_J2315 and ΔBCAM0992_K56-2, showed a significant reduction in swimming capacity. No differences in susceptibility to antibiotics or stress-inducing agents (H_2O_2, NaCl, HCl and NaOH) could be reported. Furthermore, wild type cultures of J2315 and K56-2 were supplemented with sub-MIC concentrations of sinefungin, a known DNA MTase inhibitor. As expected, supplemented cultures exhibited the same phenotype as DNA MTase deletion mutants in previous experiments.

In conclusion, our findings suggest that DNA MTases play a role in regulating the expression of genes important for various phenotypes in *B. cenocepacia* J2315 and K56-2.

Evaluation of UTP-glucose-1-phosphate uridylyltransferase (UDPG:PP) as a potential novel drug target in *Streptococcus pneumoniae*

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BACKGROUNDS - The most important virulence factor of the human pathogen *Streptococcus pneumoniae* is its polysaccharide capsule, which prevents opsonization by complement factors, adhesion and macrophage phagocytosis. Uridine diphosphate glucose (UDP-Glc) is a key component in the biosynthetic pathway of capsular polysaccharides and is also present in other bacteria where it plays a role in lipopolysaccharide and capsule production. It is formed out of glucose 1-phosphate (Glc-1P) by the enzyme UTP-glucose-1-phosphate uridylyltransferase (UDPG:PP), which is encoded by the *galU* gene. UDPG:PP is widely distributed amongst animals, plants and other microorganisms, but eukaryotic UDPG:PPs are evolutionary unrelated to their prokaryotic counterparts. Therefore, it is postulated that UDPG:PP might be a valuable novel target in fighting bacterial diseases.

OBJECTIVES – To assess the potential value of UDPG:PP in antimicrobial therapy, several *in vitro* characteristics and the *in vivo* infectivity of different pneumococcal *galU* knockout strains were compared with those of their non-mutated parent strains.

METHODS – *In vitro* data on biofilm formation, antimicrobial susceptibility and co-cultures with macrophages and epithelial cells were generated using standard methods. Transmission electron microscopy was used to visualize the capsule and *in vivo* infectivity was determined using a *Galleria mellonella* model.

CONCLUSIONS – Although there is no definitive correlation found for all strains and their knockouts, our results suggest that *galU* mutations influence *in vitro* biofilm formation. Furthermore, results of cellular co-cultures combined with the primary results of infectivity imply UDPG:PP might indeed be a potential new target.

The Syngulon PARAGEN Collection: A standardized synthetic bacteriocin gene library for rapid *in vitro* antimicrobial peptide production

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In a world where the use of broad host-killing antibiotics is becoming more of a problem than a solution, there is a critical need for alternative sources of targeted antimicrobial agents. Bacteria possess a valuable and vast reservoir of antimicrobial peptides (so-called bacteriocins) whose potential as alternatives or complements to antibiotics has yet to be realized. Recent advances in synthetic biology including the information generated by next generation sequencing (NGS) allows the exploration of the potential uses of antimicrobial peptides. One potential application is the ability to synthesize an almost unlimited collection of active and efficient bacteriocins. Syngulon has developed a synthetic biology approach to produce bacteriocins through *in vivo*/*in vitro* and chemical synthesis, paving the way to generate alternatives to antibiotics. We are developing a database (PARAGEN 1.0) of currently more than 100 nucleotidic sequences of previously described linear bacteriocins and antimicrobial peptides. Using a standardized T7-coupled transcription/translation technique we have generated a first set of *in vitro* synthesized peptides (and peptide cocktails) showing antimicrobial activity against different Gram + and Gram - strains of industrial and medical interest. Additionally, we show that peptides can be produced individually or in multiple template-containing "cocktails" without a loss of activity. This is part of our ongoing efforts in synthetic biology to increase applications of bacteriocins for tailored solutions to meet the needs of our industrial partners.

Novel cryptic prophage operon modulates stress resistance in *Escherichia coli* O157:H7

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Prokaryotic genome plasticity has paved the way for an explosive diversification within bacterial species. Part of this plasticity originates from horizontal gene transfer that takes place within the bacterial kingdom with bacterial phage genes being amongst the most common exchanged fragments. These phage genetic elements can constitute up to one fifth of the complete prokaryotic genetic blueprint but its influence on the hosts physiology remains ill defined.

Escherichia coli O157:H7 is a notorious foodborne pathogen potentiated by the large amount of horizontally transferred genomic material which differentiates it from harmless gut-inhabiting relatives such as *E. coli* K-12. Upon screening a transposon knock-out library of *E. coli* O157:H7 in search of high pressure (HP) resistant mutants, we came across a cryptic prophage encoded operon (*hpsP-hpsQ*) uniquely found in this strain that can modulate the stress resilience of the bacterial host. Disrupting the first part of this viral operon leads to a significant increase in resistance to HP as well as cross-resistance to heat with the underlying mechanism still being elusive. Upon overexpression HpsP not only proved to be a genotoxic protein with (*in vitro* and *in vivo*) DNA binding capacity, but was also able to quench the potent toxic effect of HpsQ, the second operon member. Moreover, HpsP functionality can be blocked by complementing with its own N-terminal ATPase domain thus abolishing its genotoxic DNA binding and starting host resilience modulation.

Outer membrane - peptidoglycan interactions in *Brucella abortus*

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The *Brucella* genus, part of the alpha-proteobacteria, includes Gram negative bacteria. Species of this genus are intracellular facultative extracellular pathogens and are the etiological agents of brucellosis, a worldwide neglected anthropozoonosis. *Brucella abortus* is responsible for the bovine brucellosis and can also infect humans as accidental hosts. Along the infection, *Brucella* is not only able to survive but also to proliferate within professional phagocytes (e.g. macrophages). One of the crucial factor for the short term virulence is the integrity of the outer membrane (OM).

In *Escherichia coli*, a Gram negative model bacteria, an abundant lipoprotein called Lpp or Braun's lipoprotein is found in the OM. Through the amino group of Lpp C-terminal lysine, it is covalently bound to the peptidoglycan (PG). This linkage is established by enzymes called L,D-transpeptidases (Ldts) of which 5 are found in *E. coli.* Among them, 3 are dedicated to the anchorage of Lpp to the PG, the other 2 Ldts being involved in the PG remodelling. Recently, it has been showed that Lpp is crucial to maintain a periplasmic space of constant size (Asmar *et al.*, 2017). The same study showed that altering the distance between the inner membrane and the OM could interfere with stress sensing in the envelope. There is no homolog to Lpp nor any structure establishing a link between the OM and the PG in *B. abortus.* However, multiple Ldts can be predicted in *B. abortus* genome.

To investigate the existence of OM proteins linked to the PG, we purified *B. abortus* PG while preserving proteins that could be covalently linked. We used a monoclonal antibody recognizing *B. abortus* PG to detect it by western blotting. Upon digestion of purified PG by lysozyme, bands were consistently observed at several molecular weights. Lysozyme cleaves the PG in subunits of only a few kilo Daltons. Thus, we thought that the observed bands could be due to PG fragments retained by proteins. To confirm this hypothesis, we proceeded to analysis of the digestion product through mass spectrometry (MS). Although preliminary, the MS data allowed us to pinpoint 4 proteins possibly covalently bound the PG.

Unravelling surface lipoprotein export in Bacteroidetes.

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The phylum Bacteroidetes includes saprophytes, pathogens and commensals. A hallmark of these bacteria is the presence of numerous surface-exposed multi-protein membrane complexes (Sus-like systems). These systems have a crucial role in the biology of Bacteroidetes since they allow the uptake and catabolism of a large variety of nutrients, mainly polysaccharides. Sus-like systems are predominantly composed of surface lipoproteins, which is uncommon in most studied Gram-negative bacteria while being widespread in Bacteroidetes.

Through bioinformatics analyses, we identified a lipoprotein export signal (LES) at the N-terminus of surface-exposed lipoproteins of the human pathogen *Capnocytophaga canimorsus*. We showed that, when introduced in sialidase (SiaC), an intracellular lipoprotein, this signal is sufficient to target the protein to the bacterial cell surface. We identified a LES in other Bacteroidetes, namely *Bacteroides fragilis* and *Flavobacterium johnsoniae*, suggesting the existence of a shared novel bacterial lipoprotein export pathway (1). Our current work focuses on identifying and characterizing the machinery that allows surface exposure of lipoproteins. To this aim we started different approaches based on two hypotheses:

1. Given that Sus-like systems surface lipoproteins are crucial for the uptake and metabolism of complex polysaccharides, such as starch, we can hypothesize that their depletion would be tolerated in the presence of a readily metabolizable (Sus-independent) carbon source such as glucose. Deletion of the lipoprotein export machinery would thus not be lethal in this condition. In order to identify the export machinery, we performed a random transposon mutagenesis in *F. johnsoniae* bacteria growing on glucose and then monitored the growth on starch and the starch degradation activity of single mutants. Additionally, an ELISA screen monitoring the surface localization the LES-Sialidase reporter protein was also performed on a library of 5700 transpositional mutants grown in rich medium.

2. Considering the abundance of surface lipoproteins and their crucial role in Bacteroidetes biology, we can hypothesize that the protein or protein complex responsible for lipoprotein export is essential. Therefore, we performed a transposon sequencing (Tn-seq) approach on *C. canimorsus* and *F. johnsoniae* in order to determine the set of essential genes in these species. Among the essential genes of both species we identified a gene encoding for the Omp85 family protein TamL, an homolog of TamA. While TamA is involved in autotransporter biogenesis and is found almost exclusively in Proteobacteria, TamL is a lipoprotein of unknown function exclusively present in Bacteroidetes. In order to find TamL function and in particular to see if it is involved in lipoprotein export, we are currently generating TamL conditional mutants in *C. canimorsus* and *F. johnsoniae*. Alternatively, mutants in which the export system is intentionally jammed with a known bait are also being constructed. The latter should allow to identify proteins involved in lipoprotein export by pull-down assays and co-immunoprecipitations.

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Thermodynamic characterisation of positive allosteric feedback regulation of RelA in *Escherichia coli*

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Bacteria respond to nutrient starvation by initiating the **stringent response**. The lack of nutrients is signalled by guanosine-3'5'-pentaphosphate or guanosine-3',5'-tetraphosphate, collectively referred to as **(p)ppGpp**. This signal molecule directly binds to the RNA-polymerase, changing gene expression in favour of amino-acid synthesis and downregulation of ribosome synthesis. (p)ppGpp-levels in the cells are regulated by a family of enzymes called **RSH** (RelA and SpoT Homologs). Most bacteria have one long RSH that carry out both synthesis and hydrolysis of (p)ppGpp. However, in **β- and ϒ-proteobacteria** intrinsic levels of (p)ppGpp are regulated by two antagonistic enzymes called RelA and SpoT. RelA is the main enzyme synthesising (p)ppGpp and is unable to hydrolyse it. SpoT is specialised in hydrolysis of the signal molecule.

Structural information on both proteins is lacking and their regulation is not completely understood. RelA is known to be activated by stalled ribosomes that have an uncharged tRNA in the A-site during amino-acid starvation. Additionally, (p)ppGpp was shown to have a **positive allosteric effect** on its own synthesis by RelA. Our research focusses on the molecular mechanism underlying the activity and regulation of RelA from *Escherichia coli*.

Based on isotheral titration calorimetry experiments we were able to detect and quantify binding of (p)ppGpp to $RelA_{E.coli}$. We found that (p)ppGpp binds the catalytic N-terminal domain of the enzyme, and that guanosine-3'5'-pentaphosphate has a higher affinity and consequently a stronger inducing effect on RelA compared to guanosine-3',5'-tetraphosphate. Based on the thermodynamic parameters calculated from the ITC measurements, combined with hydrogen deuterium exchange experiments we were able to predict the allosteric binding site of (p)ppGpp. This allows us to propose a molecular model describing the allosteric feedback regulation of (p)ppGpp on $RelA_{E.coli}$. These findings offer a new understanding of survival mechanisms in β- and ϒ-proteobacteria at the molecular level.

Small RNAs expressed in *Burkholderia cenocepacia* J2315 biofilms have a role in regulating iron and carbon metabolism.

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Small non-coding regulatory RNAs (sRNAs) can fine-tune developmental processes such as biofilm formation, metabolic processes such as adaption to starvation, and stress responses. In *Burkholderia* species, sRNAs are to date largely uncharacterised. Our aim was to identify and characterize sRNAs expressed in *B. cenocepacia* biofilms. After screening the whole *B. cencocepacia* J2315 biofilm transcriptome for small independently transcribed RNAs, 15 short transcripts with a pronounced secondary structure, a rho-independent terminator and a relatively high degree of conservation were designated candidate sRNAs and further investigated.

sRNA ncS63 is located in the 3'UTR of the gene encoding the hemin-uptake protein HemP, with which it shares a Fur-motif. It was strongly induced under iron-limiting conditions, and its computationally predicted targets include iron containing proteins involved in energy production, e.g. succinate dehydrogenase. This profile suggests that ncS63 has a role in iron metabolism in *B. cenocepacia*, analogous to RhyB, the sRNA regulator involved in adaptation to iron-limiting conditions in *E. coli*.

Four candidate sRNAs represent homologous sequences, found at distinct locations in the *B. cenocepacia* genome. They were more abundantly expressed in media with reduced nutrient availability compared to rich media. Each sRNA contains a CT-rich stretch which can bind to ribosome binding sites of putative targets. Predicted targets include numerous transporters for organic acids, carbohydrates and amino acids. Overexpressing one of these sRNAs caused a marked reduction, and silencing the CT-rich stretch a marked increase, in growth rate, depending on growth medium. These four sRNAs could therefore play a role in regulating substrate uptake and central carbon metabolism in *B. cenocepacia*.

sRNA ncS25 specifically targets an outer membrane porin protein with unknown function. The porin is the computationally predicted target with the highest probability and strongest interaction energy. Interaction with this target could be confirmed by qPCR and translational fusions. Overexpression of ncS25 almost completely repressed porin mRNA expression, while it had no influence on growth rate. This is analogous to porin protein-targeting sRNAs in *E. coli* such as MicA or MicF.

In summary, the *B. cenocepacia* J2315 genome contains sRNAs which appear to be part of the same regulatory circuits as those targeted by sRNAs in other bacterial species. The exact sRNA targets within these circuits and their regulatory mechanisms are specific for *B. cenocepacia*.

High ofloxacin concentrations increase persister fraction in *E. coli*

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Together with the current multidrug resistance crisis, bacterial persistence plays an increasingly important role in the failure of antibiotic treatments. Bacterial persisters are rare phenotypic variants that acquired transient tolerance to lethal doses of antibiotics. Due to their transiency and low frequency (± 1/10,000) the properties of persister cells are particularly difficult to characterize and so far, not much is known about the molecular mechanisms of bacterial persistence or the impact of antibiotic concentrations on persisters formation. Our group is especially interested in how a small subpopulation of persister cells is able to elude bactericidal action of ofloxacin, a fluoroquinolone which inhibits DNA replication by binding to DNA Gyrase. This results in double-stranded DNA breaks, ultimately leading to cell death. But before, DNA damage caused by the effect of fluoroquinolones induces the bacterial SOS response which is inhibited by the LexA repressor. Previous works showed that fluoroquinolone persisters rely on a functional SOS response, as also seen in the largely decreased rate of persisters in SOS-deficient cells (LexA3 mutant). However, the molecular mechanisms of the function of SOS and its role in ofloxacin-mediated persistence remain unknown.

In this work we investigated the impact of increasing ofloxacin concentrations on survival in exponentially growing *E. coli* cultures and found that exposure to extreme concentrations of the fluoroquinolone gave rise to more survivors than intermediate concentrations. We addressed bacterial lysis by temperate bacteriophages encoded in the genome of our reference strain and found that the lower survival in intermediate concentrations was not due to lysis of the bacteria. Since we were interested in the role of the SOS response in persister formation, we measured cellular induction of SOS by the use of a fluorescent transcriptional reporter (*sulA::gfp*) and found that the non-monotonous survival rates correlated with the level of induction of SOS. We continued our investigations on the single-cell level and observed different levels of DNA damage, caused by the different concentrations of ofloxacin. During our experiments, we came across important concentration-sensitive events on the DNA during exposure to ofloxacin, i.e. the SOS-inducible reorganization of the chromosome during fluoroquinolone treatment. Our results underline previous suggestions of an important role of the SOS response for DNA damage repair during recovery of the cells from treatment in intermediate concentrations.

Natural competence dynamics in *Streptococcus salivarius*

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In bacteria, Horizontal Gene Transfer (HGT) allow genetic exchanges between organisms. While we only start to understand how crucial those mechanisms are for bacterial evolution and adaptation, medical issues linked to HGT, such as antimicrobial resistance spread or pathogenicity acquisition, emphasize the need for new investigations.

From a bacterial point of view, human mouth is probably one of the most challenging ecological niche. Continuous physicochemical changes and competition between about 700 microbial species allow only quick-adapting bacteria to survive. In this context, HGT is essential for long-term survival. Following this strategy, Streptococcus salivarius can activate the so-called competence state, triggering bacteriocins production and efficient natural transformation. Because competence will have dramatic effects on the cell physiology, a transcriptional regulatory pathway called ComRS will fine-tune its initiation. This intricate regulatory pathway shows particularly interesting behaviour such as heterogeneous activation (bistability), and pheromone signal-mediated communication.

In this work, we investigated bistability using single-cell microscopy. Thanks to overexpressing strains of the main actors of the ComRS pathway, we were able to identify bottlenecks for the system activation. Those results underline the importance of gene stochasticity for bacterial phenotypic heterogeneous enhancement and survival at the population level.

Revealing the cell biology of a predatory bacterium in space and time

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An exciting alternative to the difficult search for new antibiotic molecules is the use of live anti-bacterial strategies naturally selected by evolution. Predatory bacteria like *Bdellovibrio bacteriovorus* invade and multiply inside the envelope of most other Gram-negative bacteria, including pathogenic strains. Upon entry in the periplasmic space of the prey envelope, *B. bacteriovorus* initiates a sophisticated developmental program in which it consumes the prey resources and grows as a polyploid filament, which eventually divides into a variable number of progeny while still inside the host. Although the potential of *B. bacteriovorus* as "living antibiotics" was demonstrated in several *in vivo* models, the molecular factors behind the exquisite biology of this micro-predator are still mysterious. Our goal is to unravel the novel mechanisms that control key processes of the cell cycle of this bacterium, using a combination of quantitative live imaging of predation at the single-cell level, bacterial genetics and molecular biology. In particular, we are interested in determining how its genetic information is copied and transmitted to the multiple progeny upon a non-binary division process, and how the predator cell is spatially organized throughout its remarkable cell cycle.

Phosphorylation of transcription factors as a signal transduction mechanism in the archaeon *Sulfolobus acidocaldarius*.

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Despite being ubiquitous and having unique and diverse metabolic capacities, Archaea constitute the least studied domain of life. Archaea were classified as Bacteria for decades until Carl Woese and his co-workers proposed a tripartite phylogenetic division composed of the domains Bacteria, Archaea and Eucarya.

Today, the existence of reversible protein phosphorylation in the three domains of life is well established and it is thoroughly studied in Eucarya and Bacteria. However, in Archaea little is still known about protein phosphorylation regarding the target proteins, their cognate kinases/phosphatases and the regulatory processes in which they are involved. As a model organism for the major archaeal family *Crenarchaeota*, *S. acidocaldarius* gains a special interest to understand signal transduction cascades and the regulatory mechanisms involved in them. Recently, a phosphoproteome study of *S. acidocaldarius* was done revealing many of the phosphorylation target proteins and sites and characterizing the role of two identified protein phosphatases; Saci_PTP and Saci_PP2A. It showed that in *S. acidocaldarius* 801 unique proteins were found to be phosphorylated *in vivo*. The phosphorylation occurs on serine, threonine and tyrosine residues and intriguingly, tyrosine was the most targeted residue.

The transcription factor $FadR_{Sa}$ (a TetR-like transcription factor encoded by *Saci_1107*that regulates a gene cluster involved in fatty acid metabolism.*)* was found to be among the phosphorylated transcription factors *in vivo*. The detected phosphorylation is occurring on the three residues Y133, T134 and T135. $FadR_{Sa}$ is

We investigated the phosphorylation event occurring in the ligand binding pocket of $FadR_{Sa}$ regarding the protein kinases involved and the possible outcome on DNA binding *in vitro* as a case study of phosphorylation regulatory function in Achaea.

Using the bioinformatics tools Archaeal Clusters of Orthologous Genes browser (arCOGs) and Archaeal and Bacterial Synteny Explorer (Absynte) we were able to identify 12 putative protein kinase genes in the genome of *S. acidocaldarius.* The putative genes were cloned in pET28b vector with N-terminal 6x Histag. Six of them were successfully expressed in *E.coli* Rosetta^{TM}(DE3) strain and the proteins were purified on His-trap column using ÄKTA FPLC system.

In vitro phosphorylation assays using [γ^{32} P]-ATP revealed that $FadR_{Sa}$ is specifically phosphorylated by the eukaryotic type-like kinases ArnC and Saci1041. Mass spectrometry analysis showed that this phosphorylation occurs only on threonine and serine residues, thus evoking the question which kinase is responsible for the tyrosine phosphorylation. Using bioinformatics tools we screened *S. acidocaldarius* kinase sequences and were able to identify sequence similarities between the putative kinases Saci1289 and Saci2317 and bacterial-type tyrosine kinase motifs. Protein purification and phosphorylation assays of both kinases are currently ongoing .
In vitro, the binding of the specific acyl-CoA ligand in the $FadR_{Sa}$ ligand binding pocket causes disruption of $FadR_{Sa}$-DNA complexes in electrophoretic mobility shift assays. However, when performing the same assay with a tyrosine phosphomimetic $FadR_{Sa}$ mutant, the protein became clearly less responsive to ligand binding(Figure 2). Thus, the tyrosine phosphorylation of $FadR_{Sa}$ in its ligand binding pocket is postulated to have a regulatory role in response to the energetic status of the cell.

Cracking the regulatory code of biosynthetic gene clusters as a strategy for natural product discovery.

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The World Health Organization (WHO) describes antibiotic resistance as "one of the biggest threats to global health, food security, and development today", as the number of multi- and pan-resistant bacteria is rising dangerously. Acquired resistance phenomena also impair antifungals, antivirals, anti-cancer drug therapy, while herbicide resistance in weeds threatens the crop industry. On the positive side, it is likely that the chemical space of natural products goes far beyond what has currently been discovered. This idea is fueled by genome sequencing of microorganisms which unveiled numerous so-called cryptic biosynthetic gene clusters (BGCs), many of which are transcriptionally silent under laboratory culture conditions, and by the fact that most bacteria cannot yet be cultivated in the laboratory. However, brute force antibiotic discovery does not yield the same results as it did in the past, and researchers have had to develop creative strategies in order to unravel the hidden potential of microorganisms such as Streptomyces and other antibiotic-producing microorganisms. Identifying the cis elements and their corresponding transcription factors(s) involved in the control of BGCs through bioinformatic approaches is a promising strategy. Theoretically, we are a few 'clicks' away from unveiling the culturing conditions or genetic changes needed to activate the production of cryptic metabolites or increase the production yield of known compounds to make them economically viable. We describe and illustrate the strategy beyond 'cracking' the regulatory code for natural product discovery, and discuss what still should be achieved to increase the rate of success of this strategy.

Molecular interactions in the phage carrier state of phage P22

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Bacteriophages, or phages, have co-evolved with their bacterial hosts for millions of years, rendering them the most ubiquitous and diversified organisms on the planet. Traditionally, two distinct reproductive strategies are described in literature. In the first strategy, the lytic proliferation, the incoming phage genome redirects the host metabolism to a virus producing machine. Phage DNA is replicated and new virions are produced, leading to the extermination of the host. For temperate phages, lysogeny is another possible development route. In this case, the incoming phage DNA is inserted into the bacterial chromosome and replicates along with it. However, both strategies display clear evolutionary disadvantages. Lytic development eventually leads to host extinction, while the lysogenic life cycle impairs progeny production. Our research group recently proposed an alternative strategy that enables phages to safely exploit their host without jeopardizing a stable co-existence. After infection, the phage genome is maintained in the host cell without immediate integration as a prophage. This 'carrier state' strategy allows the emergence of a phage-free subpopulation which is transient resistant to superinfection [1]. Further evidence for the existence of this peculiar carrier state was provided by a novel phage-host interaction that is strictly observed in phage carrier cells in the temperate phage P22-*Salmonella* Typhimurium model system. The gene product of the P22 ORFan gene *pid* derepresses the *dgoRKAT* operon of the host, which is involved in D-galactonate metabolism and important in virulence and intracellular survival [2]. In search for the molecular mechanism by which Pid derepresses the *dgoRKAT* operon and its impact on the behavior and physiology of *S.* Typhimurium, protein-protein and DNA-protein interaction studies have been performed revealing potential interaction partners involved in the regulation, expression and function of *pid*.

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High pressure SOS induction in *Escherichia coli* involves Mrr restriction endonuclease tetramer dissociation

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Escherichia coli K12 Methylated adenine and cytosine restriction protein (Mrr) is a type IV restriction endonuclease with specificity for methylated DNA. Mrr activity can be triggered by the M.HhaII Type II MTase and type III MTases of close relatives such as *E. coli* ED1A type III. In addition to MTase-dependent activation, Mrr plays a crucial role in the physiology of *E. coli* after high hydrostatic pressure (HP) shock. A sub-lethal HP treatment of 100 MPa triggers Mrr activity causing double-strand breaks in the chromosome and leading to SOS response induction. In this work, we first investigated the mechanism of HP and MTase dependent activation *in vivo* using Fluorescence Fluctuation Microscopy. Analysis of Mrr by Number and Brightness using a validated GFP-Mrr fusion protein demonstrate that Mrr is a tetramer in unstressed cells and shifts to a dimer upon HP shock or co-expression with M. HhaII. Based on differences in reversibility of tetramer dissociation observed for wild-type GFP-Mrr upon HP shock compared to M.HhaII expression, a model for Mrr activation is proposed in which HP triggers Mrr activity by directly pushing inactive Mrr tetramers to dissociate into active Mrr dimers, while M.HhaII triggers Mrr activity by creating high affinity target sites on the chromosome, which pull the equilibrium from inactive tetrameric Mrr toward the active dimer. In order to identify a potential Mrr recognition consensus sequence *in vivo*, a chromatin affinity purification assay coupled with cloning and sequencing is currently being developed.

Nucleotide binding changes the activity of the universally conserved GTPase ObgE

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Although essential for bacterial viability, the cellular functions of the universally conserved GTPase Obg remain enigmatic. Moreover, the influence of GTP and GDP binding on the activity of this essential protein is largely unknown. Previously, we identified a mutant isoform of ObgE (the Obg protein of Escherichia coli) that triggers cell death. Using cell death as an easy read-out for protein activity, we explore the structural and biochemical requirements for the toxic effect of this mutant ObgE* isoform. Both the absence of the N-terminal domain and the inability to bind GTP neutralize ObgE*-mediated toxicity. Moreover, a deletion in the β-strand that connects the N-terminal domain to the G domain can likewise abolish ObgE*-mediated toxicity. Taken together, these data indicate that GTP binding by ObgE* triggers a conformational change that is transmitted to the N-terminal domain to confer toxicity. We therefore conclude that ObgE*-GTP, but not ObgE*-GDP, is the active form of ObgE* that is detrimental to cell viability. Based on these data, we strongly suspect that also for wild-type ObgE, GTP binding triggers conformational changes that affect the N-terminal domain and thereby control ObgE function.

Directed evolution; From plasmid library to genomic library

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Previously in our team, one of the attempts to evolve DD-peptidase to beta lactamase, led to partial improvement of DD-peptidase ability to destroy penicillin but, at the same time, it appears very toxic for the host bacterium. This suggests that the evolutionary trajectory linking the two enzymes is a very sharp pathway. To date, the directed evolution of genes is mostly performed in **plasmids**, that are the most-commonly used cloning vectors and convenient to handle but lead to large amounts of enzymes, which can be a critical issue if the enzyme is toxic. Currently, we are applying the recent powerful genome editing technology, CRISPR-Cas9, to deliver our library of variants directly into **genome** of bacteria instead of plasmids, which is much closer to the natural way of gene evolution and also producing less amount of enzymes towards a less fitness effect.

Streptococcus thermophilus, a powerful platform to generate massive gene libraries

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Determining the sequence-function relationship of a protein is one of the fundamental challenges of structural biology. A recently developed method called deep mutational scanning (DMS) allows us to explore the functional effects of billions of mutations simultaneously. This method combines the explorative competence of large-scale protein variant libraries, with the power of high throughput sequencing, allowing us to link subtle changes in the phenotype of the variant to its genotype.

One of the factors for the effectiveness of DMS relies on our capacity to create expansive gene libraries, with genes under selection in uniform expression conditions. Traditionally, plasmids have been used as convenient genetic elements for creating gene variant libraries. However, expression from variable-copy number plasmids may not faithfully replicate the phenotype of proteins that are expressed from a chromosomal locus, and lead to variety in expression levels. To this aim, we decided to leverage the natural competence of *Streptococcus thermophilus* to create massive chromosomal gene libraries of ~10^9 variants.

Our method could be used to study the biological significance of variation in genes associated with genetic disease, to design better starting points for directed evolution of enzymes or to explore fundamental questions about molecular evolution.

Molecular and structural characterization of a novel transcription factor from the GntR transcription factor family in Sulfolobus acidocaldarius

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Transcription factors (TFs) of the GntR family regulate many physiological and morphological processes in Bacteria, often in response to the nutritional state (1) (2). Based on their variable C-terminal domain, these TFs are subdivided in several subfamilies, including the YtrA subfamily, which is characterized by a small C-terminal domain. In Bacteria, YtrA TF-encoding genes are often located in the neighborhood of genes encoding transport proteins which are, as far as described, involved in antibiotic production and resistance, carbohydrate uptake or a type III Secretion system (3) (4) (5) (2) (6). Despite their wide distribution amongst bacterial species, no archaeal GntR-type regulator has been described yet. Here, we present the characterization of the first archaeal GntR/YtrA-like TF in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*, named SaYtrA.The crystal structure of SaYtrA was determined to a resolution of 2.6 Å. The protein formed a dimer within the crystals. SaYtrA protein displays specific binding *in vitro* and *in vivo* to a DNA probe harbouring the control region of an operon encoding the SaYtrA regulator itself and a putative membrane protein. DNaseI footprinting revealed a 25 bp semi-palindromic recognition binding site covering the transcriptional start. This binding site sequence was used for an *in silico* screening of the genome sequence, leading to the prediction of additional target genes systematically tested by *in vitro* and *in vivo* binding assays, revealing a genomically distant gene encoding another putative membrane protein. A reverse transcriptase PCR (qRT-PCR) comparing a *SaYtrA* overexpression strain with its isogenic wildtype showed downregulation of both targets when SaYtrA is upregulated. In conclusion, SaYtrA represses transcription of two putative membrane protein-encoding genes.

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Characterization of the molecular mechanisms controlling negative chemotaxis to Cu in *Caulobacter crescentus*

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Pathogenic, symbiotic and free-living bacteria are likely to be exposed to toxic concentrations of heavy metals, such as copper (Cu), requiring a rapid response to maintain their fitness. We have previously shown that the dimorphic alphaproteobacterium *Caulobacter crescentus* uses a bi-modal strategy to cope with toxic Cu concentrations, where the sessile stalk progeny triggers Cu oxidation and efflux within a few minutes whereas its flagellated sibling rapidly flees towards a Cu-free environment (Lawarée *et al.*, 2016).

The molecular mechanisms underlying bacterial chemotaxis upon heavy metals exposure have remained unknown so far. A high cellular Cu concentration is essential for *Caulobacter* negative chemotaxis, suggesting that a Cu sensory system monitors intracellular variations of Cu concentrations. A bioinformatics analysis reveals the presence of 19 chemoreceptors (MCPs – Methyl-accepting Chemotaxis Proteins) encoding genes in *C. crescentus* genome. The invalidation of the 19 genes separately led us to identify four candidates (McpC, G, P and R) displaying a reduced Cu flight, suggesting a potential redundancy or cooperation in the control of negative chemotaxis to Cu. Double and triple mutants of these candidates will be further generated to address this hypothesis. Despite the absence of a typical Cu-binding CXXC region in McpC, G, P and R, McpR sensory domain displays a □□□□□□ fold, which has been shown to bind Cu in other bacterial species.

Although still under debate, Cu has been associated to the generation of an oxidative stress. A genetic screen of a transpositional mutants library realized in our lab identified mutants impaired in oxidative stress regulation and exhibiting a reduced Cu flight. One may hypothesize that negative chemotaxis relies on a biphasic sensing by both Cu-sensitive MCP and ROS-sensitive MCP. In order to address this hypothesis, we are currently investigating the chemotactic behaviour of (i) SOD and catalase mutants exposed to Cu and (ii) MCP mutants exposed to H_2O_2. The preferential accumulation of Cu in the periplasm measured by inductively coupled plasma optical emission spectrometry (ICP-OES) in cell fractionates suggests that Cu/ROS sensing may occur in the periplasm.

The persistent role of toxin-antitoxin systems in antibiotic tolerance

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Persistence is widely acknoweldegded as a phenotypic switch by which a sub-percent fraction of a bacterial population is able to survive an antibiotic treatment. This switch has been attributed to the ability of bacteria to stochastically enter a slow or non-growing state in which antibiotics have no effects. For a long time, the idea that toxin-antitoxin systems could induce this growth arrest as a result of toxin level fluctuations was widely accepted. In particular, type II toxin antitoxin (TA) systems of Escherichia coli, in which the toxic components are translation-inhibiting mRNAses, were shown to be a requirement for persistence. Previously published data showed that successive deletions of 10 TA systems progressively reduced persistence up to 100 fold (1). Moreover, fluorescent reporters of TA transcriptional activity showed that these systems were induced in a small fraction of drug-tolerant cells, showing that TA systems were indeed the cause of persistence (2).

We tried to replicate these experiments and showed that the constructs used in these previous studies were not adequate. The TA deletions mutants were infected by phages as previously described (3). A new Δ10TA mutant constructed by our lab showed persistence levels similar to the wild-type strain, both for ampicillin and ofloxacin. We also showed that TA transcriptional reporters used in these studies were not functional and that the authors probably tracked stochastic increases in autofluorescence. We redesigned these reporters and imaged single persister bacteria by live imagining during antibiotic treatment. Surprisingly, persister cells showed no significant changes in reporter intensity compared to the bulk of the population (4). Altogether, these results underline the limitations of previously published experiments and show that type II TA systems are not implicated in persistence in E. coli.

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Two-component systems in *Streptococcus salivarius:* signal transduction controlling natural competence

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Streptococcus salivarius (*Ssa*) is a commensal bacterium and a major player of the human oral microflora, which mainly colonizes epithelia by forming biofilms. Besides highly variable physico-chemical parameters, the oral cavity is one of the most competitive ecological niche since at least 700 microbial species coexist and interact through potential synergistic or inhibitory effects. Therefore, species dwelling in the mouth undergo a strong selective pressure that imposes a constant evolution and adaption. In this context, horizontal gene transfer is an asset in order to ensure their long-term survival. As most genome-sequenced streptococci, *Ssa* encodes all essential competence genes required for natural DNA transformation. In *Ssa*, this mechanism responsible for genomic plasticity is fully functional and enables chromosomal integration of exogenous DNA.

This work aims to characterize physiological responses of *Ssa* to variations in environmental parameters taking place in the oral cavity. Considering their sensing role, we focus on the involvement of Two-Component Systems (TCSs) on (i) competence activation cascade, (ii) biofilm formation - the presumably prevailing mode of growth in the mouth - and (iii) production of antimicrobial molecules (predation mechanism).

In silico analysis revealed that *Ssa* encodes 14 TCSs (13 pairs of Histidine Kinases (HK) and Response Regulators (RR) and one orphan HK). In a global survey, we systematically inactivated each TCS (gene replacement strategy) in luminescent reporter strains to screen promoter activity of genes required at several stages of competence state (*comR*, *comX* and *dprA*). While 3 systems are likely to be essential, we showed that the deletion of 3 TCSs (out of 11) influences the competence state: CiaRH, LiaSR and HK15. Markedly, we observed that the HK15 inactivation impedes natural transformation, presumably by reducing ComR steady-state levels. Coherently, it also hinders bacteriocin production, a ComR-dependent predatory behaviour. Further results should give us a better understanding on how environmental stimuli impact on the acquisition of exogenous DNA and related molecular processes (development, antimicrobial peptides secretion,…) in *Ssa*. Those mechanisms are usually interconnected and primordial for species survival and adaption.

Toxin-antitoxin system settled in criptic prophage and regulated in a lambda fashion

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The role of prophages in bacteria exceeds the sole purpose of the phage and present reservoir of genes beneficial to bacteria. Prophages constitute significant proportion of *E. coli* genomes and participate in evolution and virulence of pathogenic strains through active rearrangements. One way to secure stable inheritance is through phenomenon known as post-segregational killing exerted by toxin antitoxin (TA) systems. Toxin-antitoxin systems are typically small two-component operons coding for toxic protein and its antidote. Toxic proteins under normal conditions are neutralized by their cognate antitoxins. In type II TAs both components are proteins and form strong complex which sequesters the toxin. Such complex will also bind to the operator region of the TA operon and repress its transcription. The regulatory abilities are exerted through DNA-binding domains typically located in the N-terminus of the antitoxins. However, several cases of tripartite TA modules, arranged as regulator-antitoxin-toxin, have been reported where transcription regulation is orchestrated by a separate third component. *PaaR2-paaA2-parE2* system from *E. coli* O157:H7 encodes replication inhibiting toxin related to the gyrase poison $ParE_{RK2}$. Because of existence of separate regulator this system defies typical auto-repression mechanism observed for TA systems that relies on formation of complexes of different ratio of antitoxin and toxin. *paaR2-paaA2-parE2* tripartite system is located in CP933-P prophage. CP933-P (also known as Sp12) is one of the putatively defective prophages in O157:H7 strains which was reported to be actively rearranged, however the central region, comprising TA system, was shown to be stable. We took a closer look at the genetic context of the *paaR2-paaA2-parE2* locus and therefore question the idea of tripartite TA system. We have shown that it is in fact regulated by several other regulators in addition to PaaR2. We demonstrated that *paaA2-parE2* toxin-antitoxin genes are embedded in an intricate lambdoid prophage-like regulation network. We have acquired *in vivo* and *in vitro* evidence that PaaR2 regulator is a functional homologue of lambda repressor CI. Further, the neighbouring operon encodes functional homologues of Cro and CII respectively, that are crucial for transcription regulation of *paaR2-paaA2-parE2* operon and their own operon. Using single cell analysis and time-lapse microscopy we have observed that the entire locus exhibits bistablity which generates diversity of expression in the population. Taken together we demonstrate that *paaA2-parE2* toxin-antitoxin genes have fallen into the regulatory hotspot of the cryptic prophage which can be beneficial for both TA system and a prophage itself.

Structural dynamics of the non-folded, translocation-competent secretome

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Sec-dependent secretory preproteins carry N-terminal signal peptides and are targeted post-translationally to the translocase complex SecYEG, while being maintained in non-folded states. Upon translocation, the signal peptide is cleaved and folding into mature native structures initiates (Tsirigotaki et al., 2017). Factors that delay folding in the cytosol are known but not the molecular basis of their effect. Both the type of signal peptide, the linkage to mature domain, the nature of the early mature domain and chaperones affect folding states (Sardis et al., 2017; Tsirigotaki et al., 2018). An additional feature is intrinsic to mature domains: they predominantly display slow folding and retain loose, long-lived intermediates. These properties emerge from residue composition, elevated disorder and reduced hydrophobicity (Tsirigotaki et al., 2018) and distinguish secreted proteins as a protein class, distinct from cytoplasmic folders.

To address what structural elements impact protein trafficking and folding, we analyse secretory proteins using hydrogen-deuterium exchange mass spectrometry (HDX-MS) and single molecule FRET (Förster Resonance Energy Transfer). With HDX-MS, the rigidity and overall structural dynamics of protein folding states are established by monitoring the exchange between amidic protons on the polypeptide's backbone with deuterium provided in the solution. smFRET analysis determines distance changes between fluorophore-labelled amino acid pairs in the sequence. As the polypeptide undergoes folding transitions, smFRET can determine folding intermediates, inter-conversion kinetics between states and lifetimes of states as well as chaperone binding.

We are focusing on several secretory proteins including maltose binding protein (MBP) and the prolyl cis-trans isomerases PpiA and PpiB as model proteins. HDX-MS on MBP has revealed distinct folding intermediates and these can be followed by smFRET. The native MBP structure folds in several minutes, whereas the presence of a signal peptide kinetically slows down folding. In contrast, the chaperone SecB alters the folding pathway by stabilizing more loose folding intermediates. Periplasmic PpiA and cytosolic PpiB are structural homologues, but show different HDX-MS-detectable folding kinetics. PpiA folds significantly slower than PpiB by retaining a conformationally loose intermediate state (Tsirigotaki et al., 2018). Extending this analysis, we are developing a smFRET pipeline to track the conformational dynamics of PpiA/B intermediates. Moreover, bioinformatics and structural analyses have led to the prediction of PpiB residues important for fast folding and these are being tested by site-specific mutations.

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Monitoring protein secretion in *Streptomyces* using fluorescent proteins

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Fluorescent proteins are a major cell biology tool to analyze protein sub-cellular topology. Here we have applied this technology to study protein secretion in the Gram-positive bacterium *Streptomyces lividans* TK24, a widely-used host for heterologous protein secretion biotechnology. Green and monomeric red fluorescent proteins were fused behind Sec (SP^{Sec}) or Tat (SP^{Tat}) signal peptides to direct them through the respective export pathway. Significant secretion of fluorescent eGFP and mRFP was observed exclusively through the Tat and Sec pathways, respectively. Plasmid over-expression was compared to a chromosomally integrated *sp^{Sec}-mRFP* gene to allow monitoring secretion under high and low level synthesis in various media. Fluorimetric detection of SP^{Sec}-mRFP recorded folded states, while immuno-staining detected even non-folded topological intermediates. Secretion of SP^{Sec}-mRFP is unexpectedly complex, is regulated independently of cell growth phase and is influenced by the growth regime. At low level synthesis, highly efficient secretion occurs until it is turned off and secretory preforms accumulate. At high level synthesis, the secretory pathway overflows and proteins are driven to folding and subsequent degradation. High-level synthesis of heterologous secretory proteins, whether secretion competent or not, has a drastic effect on the endogenous secretome, depending on their secretion efficiency. These findings lay the foundations of dissecting how protein targeting and secretion are regulated by the interplay between the metabolome, secretion factors and stress responses in the *S. lividans* model.

Keywords:

eGFP, mRFP, protein secretion, signal peptide, *Streptomyces lividans*, protein secretion biotechnology

ABSTRACTS POSTERS

SECTION B: APPLIED AND ENVIRONMENTAL MICROBIOLOGY

Ability of plastic-associated bacterial community to degrade petro-based plastics in marine environment

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Because of the essential role of plastics in human life, the production of plastics increases every year. Most plastics are released to the environment in landfills and end up in the sea, inducing large ecological and health impacts. Plastics constitute a physical substrate and potential carbon source for microorganisms. However, little is known about the microbiomes associated to these plastics and plastic biodegradation. The present study compares the structures of bacterial communities from floating plastics, sediment-associated plastics and sediments from the Mediterranean Sea (Stareso, Corsica). The 16S rRNA microbiome profiles of surface and sediment plastic-associated microbial biofilms from the same geographic location differ significantly, with the omnipresence of Bacteroidetes and Gammaproteobacteria. Second, the present study uses environmental samples to investigate the enrichment of potential plastic-degrading bacteria with Low Density PolyEthylene (LDPE), PolyEthylene Terephthalate (PET) and Polystyrene (PS) plastics as the sole carbon source. *Alcanivorax borkumensis* and *Microbulbifer* sp. appear to be enriched in medium with LDPE, and the LDPE surface shows signs of degradation. Interestingly, other hydrocarbon-degrading bacteria, such as *Marinobacter* and *Arenibacter,* are also enriched with LDPE and PET as carbon sources, implying that these bacteria are potential players in plastic degradation.

Upgrading wastewater resources to microbial protein: Maximizing protein production of purple bacteria and assessing its application on sewage

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Background. The conventional fertilizer–food chain is a cascade of inefficiencies. Upgrading nutrients from wastewater to microbial biomass could improve the efficiency, with this protein-rich product used as ingredient in animal feed preparations. It is a challenge to produce a biomass on wastewater with a highly controllable quality, given its diversity and complexity in organics composition. Anaerobic fermentation might render a solution, through its generation of a more simple mix of VFA. PNSB cultivated in a photoheterotrophic mode provide an excellent target group, as they possess a virtually perfect carbon assimilation efficiency. To date, fermentation research focusses on directing the carboxylates to particular VFA or to a mix. For now, there is still no understanding how the type of VFA affects PNSB production. That is why the objective of this study was to map the effects of individual and mixed VFA on PNSB growth kinetics, carbon yield and protein production to maximize nutrient recovery. In addition, a case study for the EU-28 was performed to assess the market potential of sewage organics as source of PNSB.

Methods. Batch cultivations were performed with three different VFA (acetate, propionate, butyrate) and with a mix of these compounds (1/1/1 on C-basis). The microorganisms used for the experiments were *Rhodopseudomonas palustris, Rhodospirillum rubrum* and *Rb. sphaeroides* along with a synthetic community (mixture of the three strains 1/1/1 volatile suspended solids ratio) and an enrichment community, obtained from incubating sewage activated sludge and sediment from a local pond (1/1 volatile suspended solids ratio) with a VFA mix based medium. All tests were performed under photo-anaerobic (30W/m² light intensity) conditions at 28°C. The microbial community was characterized by 16S sequencing (V4 region; Illumina MiSeq). To assess the potential of sewage organics as source of PNSB, a three-step methodology was used: i) Calculate the amount of sludge that can be captured from sewage organics for primary settling, chemically enhanced primary treatment (CEPT), high-rate conventional activated sludge (HiCAS) and high-rate contact stabilization (HiCS) all followed by activated sludge as polishing stage. ii) Calculate the amount of VFA that can be fermented from the captured sludge. iii) Calculate the amount PNSB that can be upgraded from VFA. Monte Carlo simulations with 10,000 iterations were uses to account for uncertainty (technological performance, COD influent load and market value).

Main results. Results showed that the growth rate of the pure cultures and the enrichment community was elevated between 1.3-2.5 times for the VFA mix compared to the single VFA compounds. The carbon yield for all experiments was around 1 g $COD_{biomass}$ g-1 $COD_{removed}$ and there was no difference between different PNSB and the source of organic. Overall, the enrichment community performed best, with superior growth rates (1.8–2.2 d^{-1}). The case study for the EU-28 showed that upgrading VFA would allow to produce 0.6-2.2 megatonnes of microbial protein, substituting 21-72% of the global fishmeal production.

Take-home messages. This study demonstrated that a VFA mixture will positively affect the PNSB production kinetics and stoichiometry as well as product characteristics. Best results were obtained with *Rb.sphaeroides* and the enriched *Rb.capsulatus*. Moreover, the case study performed for the EU-28 shows that sewage organics have a great potential as source for microbial protein and can substitute over 55% of current fishmeal protein production. However, technological developments ought to be supported by health safety of the final product and a legal framework for microbial protein derived from sewage organics should be constructed.

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Vagococcus isolates from marine sponges inhibit growth of multi-resistant *Staphylococcus aureus* strains

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Staphylococcus aureus is a common bacterial pathogen, responsible for infectious diseases worldwide, especially difficult to treat when caused by methicillin-resistant *S. aureus* strains. There is now an important need for the discovery and development of antibiotics. In this context, marine sponges have been attracting interest as a source of bioactive compounds. Particularly, sponge-associated bacteria have been shown to be responsible for the production of many compounds with pharmacological functions, such as antibiotics.

Sponge-associated bacteria isolated from two sponge species (*Hymeniacidon perlevis* and *Halichondria panicea*) collected in 2013 at Wimereux (FR) were studied for their antibacterial activity against *Staphylococcus aureus* strain ATCC29213 using a double layer antagonism test. Over 50% of all isolated bacteria inhibited the growth of *S. aureus*. The most interesting isolates with antibiotic activity belonged to the genus *Vagococcus*, which has not been reported in the literature for antibacterial activity.

Vagococcus is a genus belonging to the lactic acid bacteria or Lactobacillales order. To ensure that the lactic acid bacteria's acid production was not responsible for the antibacterial activity, the antagonism test was performed with culture pellets washed with sterile water. The antibacterial activity was unchanged compared to unwashed pellets, proving that the acid in the culture medium was not responsible for said activity.

A collection of multi-resistant *S. aureus* strains isolated from infections in hospitals and with diverse antimicrobial susceptibility profiles, including resistance to all families of antibiotics tested, was used to further test the antibacterial activity of the five *Vagococcus* isolates. All isolates inhibited the growth of 57 to 86% of the multi-resistant *S. aureus* strains. The results of those experiments suggest that our five *Vagococcus* strains, despite identical 16S rRNA gene and similar growth curves, do not produce the same antibiotic compound(s) as they do not harbor identical activity profiles against the 37 *S. aureus* strains.

The spectrum of activity of the *Vagococcus* isolates will be further studied, testing said activity against other bacterial strains for which the need for new antibiotics is critical. The antibiotic compound(s) produced by the *Vagococcus* isolates will be isolated and identified as they show great potential as antibiotic agents against multi-resistant *S. aureus* strains.

EX-SITU CONSERVATION and explorATION of POLAR cyanobacteria IN The BCCM/ULC Collection

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The BCCM/ULC public collection funded by the Belgian Science Policy Office since 2011 aims to gather a representative portion of the polar cyanobacterial diversity with different ecological origins (limnetic microbial mats, soil crusts, cryoconites, endoliths, etc.). It makes it available for researchers to study the taxonomy, evolution, adaptations to harsh environmental conditions, and genomic make-up. It presently includes 174 cyanobacterial strains, with more than half being of polar origin (catalogue: http://bccm.belspo.be/catalogues/ulc-catalogue-search).

The morphological identification shows that the strains belong to the orders Synechococcales, Oscillatoriales, Pleurocapsales, Chroococcidiopsidales and Nostocales. The large diversity is also supported by the phylogenetic analyses based on the 16S rRNA sequences. This broad distribution makes the BCCM/ULC collection particularly interesting for phylogenomic studies. As an example, to better understand the survival strategies of an Antarctic cyanobacterium, we have determined the genome sequence of the axenic strain *Phormidesmis priestleyi* ULC007 by High Throughput Sequencing and investigated the abundance of genes in targeted functional categories based on the RAST subsystems technology.

In addition, cyanobacteria produce a range of secondary metabolites (e.g. alkaloides, cyclic and linear peptides, polyketides) with different bioactive potential. Bioassays have shown antifungal activities of the cell extracts of strains *Plectolyngbya hodgsonii* ULC009 and *Phormidium priestleyi* ULC026. Due to the geographic isolation and the strong environmental stressors of the habitat, the exploration of these metabolites in Antarctic cyanobacterial strains seems promising for biotechnology or biomedical applications.

BCCM/ULC obtained an ISO 9001:2015 certification for public and safe deposits, and for distribution of living strains and genomic DNA. The BCCM policy continuously aims to guarantee a safe fit-for-use microbiological material and data compliant with the rules on access and utilization of the Nagoya Protocol. In addition, BCCM/ULC provides, to clients from academia & industry, a service of morphological identification and molecular characterization, along with other scientific services as tailor-made trainings and collaborations.

Optimization of protease adsorption from *Bacillus licheniformis* on bentonite and supplementation in poultry feed

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Abstract

Alkaline proteases are widely used in diverse fields such as food, textiles and detergent industry. In the current study, an alkaline protease was subjected to immobilization to investigate its potential use as a feed supplement in poultry. The enzyme was produced from *Bacillus licheniformis* PB1 under pre-optimized conditions and partially purified using ammonium sulfate precipitation at 80% saturation, yielding 8.21-fold increased activity with 59% enzyme yield. Furthermore, immobilization support material and experimental conditions such as buffer molarity, pH and temperature were optimized. The highest increase in free enzyme activity (a maximum of 19.02-fold) was observed using an optimal reaction temperature of 50℃, 1M potassium phosphate at pH 8. Subsequently, after immobilization on bentonite the stability of alkaline protease towards substrate greatly enhanced. The adsorbed alkaline protease retained 86.39±4.36% activity under optimized parametric conditions. Additionally, dried immobilized enzyme after two weeks storage at room temperature exhibited only 5% activity loss. Eventually, protease immobilization on bentonite facilitates longer storage at room conditions and the dramatic hygroscopic nature of bentonite reduced protein denaturation upon drying. Application of absorbed product helped broilers gain weight significantly (ANOVA, P=0.001) when fed along with basal feed 46 days in comparison to the commercially available enzyme supplement and control for.

Keywords

Bentonite, Carlsberg Subtilisin, Immobilization, Solid support, Poultry Feed supplementation

RADIATION RESISTANCE IN THE CYANOBACTERIUM *ARTHROSPIRA*

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The multicellular cyanobacterium *Arthrospira* has been studied for many years because of its excellent nutritive value as a food- and feedstock and its many applications in biomedical sciences. The MIC group at SCK•CEN studies *Arthrospira sp. PCC 8005* as a principal organism and edible endproduct of the MELiSSA bioreactor, a life support system developed by the European Space Agency. Our aim is to elucidate the genetic and biochemical pathways involved in the resistance of strain *PCC 8005* to acute high doses of gamma radiation, i.e, upto 5,000 Gy, and the fundamental principles of cellular radiation resistance. To study the effect of ionizing radiation (IR) on the growth and morphology of *Arthrospira* and to check whether resistance to extreme high IR doses is a general trait in *Arthrospira*, different strains of *Arthrospira* were exposed to increasing doses of Co^{60} gamma radiation. They were analyzed for culture-based growth recovery, morphological changes, and cellular and molecular effects. For the latter we used TEM microscopy and LC-MS/ESI-TOF metabolic profiling. We also studied these strains for IR-induced changes in the intracellular content of proteins, pigments, carbohydrates, and fatty acids. We found that resistance to IR in *Arthrospira* strains is not just confined to the strain *PCC 8005* but this feature was also present in all other *Arthrospira* strains investigated, albeit that some strains seemed to be more resistant to IR than others, i.e. the lethal dose varied in the range of 2,000 – 5,000 Gy. We also observed during continuous cultivation of strain *Arthrospira sp. PCC 8005* under controlled conditions that slight variations in growth conditions can have a profound effect on its trichome morphology, with a permanent change from the original spiral form to a straight morphotype. In concordance, we noted differences between these morphotypes in the amount of light harvesting antenna, the cellular content of hydrophilic and lipophilic proteins and fatty acid composition, although the FTIR analyses suggest that organic chemical compositions of those morphotypes are analogous. Interestingly, the new straight trichome morphotype was found to be more resistant to IR than the original spiral morphotype. To further understand this complex response, we performed an in-depth analysis using mass spectrometry and are designing experiments to follow changes in gene expression using RNAseq. In a later stage, a resequencing project is planned to elucidate the genetic changes between the spiral and straight morphotypes.

Relationship between redox state and polyhydroxyalkanoates production in *Rhodospirillum rubrum* : metabolic insight and copolymers production

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Abstract

Rhodospirillum rubrum is a purple non-sulphur bacterium (PNSB) belonging to the α-proteobacteria group, well-known for its metabolic versatility. This photosynthetic bacterium is capable of assimilating broad range of carbon sources including VFAs and hence could be grown under both autotrophic and heterotrophic conditions. *Rhodospirillum rubrum* is able to perform aerobic respiration as well as anoxygenic photosynthesis using light as energy source and carbon sources as electron source. Among those carbon sources, volatile fatty acids (VFAs) are extensively studied due to their cheapness. Effectively those compounds resulting from fermentation processes are found for example in wastewater treatments effluent, activated sludge, or brewery. Since these compounds are a waste effluent source, their use for large scale cultivation could bring down the input costs, biotechnological processes such as polyhydroxyalkanoates production are expected to be more economically favourable using those wastes.

PHAs are used as carbon or energy storage inclusions when lack of a nutrient (N, S, P) impairs bacteria growth. However, in our case, PHA production by *Rhodospirillum rubrum* is not induced by the lack of nutrient but more by a unbalanced redox state as it has been observed in *Synechocystis* PCC6803. This redox stress could be explained by the use of reduced substrates as VFAs or light intensity. In this case, the PHAs production could be used as an electron sink by the bacterium.

Several studies have already shown that the PHA composition is driven by the carbon sources provided in the environment. This discovery boosted the lure of PHA. Whereas PHB are stiff, brittle and shows high degree of crystallinity, P(HB-*co*-HV) are flexible and possess low crystallinity, tensile strength and high melting point. Whereas the assimilation of acetic acid (C2) or butyric acid (C4) are most of the time related to the production of pure PHB, the use of C3 (*e.g.* propionic acid) or C5 (*e.g.* valeric acid) is often related to the production of P(HB-*co*-HV) copolymer with high HV monomer percentage.

In this research we investigated the link between the redox stress and polyhydroxyalakanoates and the link between carbon source and the type of PHA produced. In order to do so, we trigger different type of redox stress by using reduced carbon source and increasing light intensities to trigger light induced stress.

EPS production by Cyanobacteria: First insight in *Cyanothece sp*. PCC 7822 metabolism

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The present research is a part of the Algotech project based on high-added value compounds production by Microalgae/Cyanobacteria. In this context, we investigate exopolysaccharide production by Cyanobacteria because of their properties and applications in cosmetics, medicine or food industries. In addition, the use of these organisms for EPS production has numerous positive aspects compared to microalgae or plants as high growth rate, easier manipulation by genetic engineering or economical costs of production.

Cyanothece sp. PCC 7822, an unicellular diazotrophic cyanobacterium, was selected due to its ability to produce high rate of EPS. This study focuses on the impact of culture conditions on the bacterium metabolism and EPS production. Four different N sources ($NaNO_3$, NH_4Cl, Urea and atmospheric N_2 at 17 mM in term of N) are tested in continuous light or in 12h/12h light/dark cycle.

First results indicate that $NaNO_3$ as N source induces the best growth in the 2 light conditions tested. The strain is also able to growth in presence of ammonium but not urea even if urease genes are presents in the genome. Observation of EPS by alcian blue staining indicates the presence of carboxylic and sulphated groups in their composition. Variation of EPS configuration according light parameters and N sources is highlighted which could be related to changes in exopolysaccharide composition. Moreover, EPS configuration seems also to change over the growth in a same condition. Furthermore, pigments profile has been done in order to characterize the strain PCC 7822 and standardize EPS concentration.

In the frame of a project implicated in microalgal energetics and biomass promotion, this work targets the EPS production by *Cyanothece sp*. PCC 7822 by using innovative processes. This bacterium *Cyanothece sp*. PCC 7822 shows its full potential by its EPS composed of carboxylic and especially sulphated group which is not the case with microalgae. In fine, this particular composition leads to saccharides with interesting industrial properties.

Dereplication of MALDI-TOF MS spectra via the "SPeDE" algorithm.

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Microorganisms are the most diverse group of life present on earth and are ubiquitous. Traditionally this diversity has been studied via standard microbial plating techniques. The development of cultivation independent technologies led to a revolution in microbial diversity insights. It has become clear that in many biological samples a large proportion of microbial cells cannot be cultivated under standard laboratory conditions, a phenomenon known as the "great plate count anomaly". This makes the isolation and cultivation of the "biological dark matter" a topic of great interest, and may lead to a so-called cultural revolution. However, standard plating techniques are not yet fully deployed to reach the maximum diversity possible. For example, simply altering cultivation conditions still reveals new microbial diversity. We aim to develop and test in a proof-of-concept manner a high-throughput semi-automated, miniaturized culturomics pipeline. A key step in this process is the dereplication of the large number of isolates obtained into a small, non-redundant set of operational isolation units (OIUs). An OIU consists of a set of isolates considered the same at the level of dereplication and is represented by a reference isolate. Here, we present the "SPeDE" algorithm which we developed to dereplicate large numbers of MALDI-TOF MS spectra in a time efficient manner. Currently, we are able to process and analyze up to 5,000 isolates per week. The results of a proof of concept study on 10,000 isolates derived from a single forest soil sample will be presented.

Isolation, characterization, and antibacterial activity of hard-to-culture Actinobacteria from cave moonmilk deposits

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Cave moonmilk deposits host an abundant and diverse actinobacterial population that has a great potential for producing novel natural bioactive compounds. In our previous attempt to isolate culturable moonmilk-dwelling Actinobacteria, only *Streptomyces* species were recovered, whereas a metagenetic study of the same deposits revealed a complex actinobacterial community including 46 actinobacterial genera in addition to streptomycetes. In this work, we applied the rehydration-centrifugation method to lessen the occurrence of filamentous species and tested a series of strategies to achieve the isolation of hard-to-culture and rare Actinobacteria from the moonmilk deposits of the cave "Grotte des Collemboles". From the "tips and tricks" that were tested, separate autoclaving of the components of the International Streptomyces Project (ISP) medium number 5 (ISP5) medium, prolonged incubation time, and dilution of the moonmilk suspension were found to most effectively improve colony forming units. Taxonomic analyses of the 39 isolates revealed new representatives of the *Agromyces*, *Amycolatopsis*, *Kocuria*, *Micrococcus*, *Micromonospora*, *Nocardia*, and *Rhodococcus* species, as well as additional new streptomycetes. The applied methodologies allowed the isolation of strains associated with both the least and most abundant moonmilk-dwelling actinobacterial operational taxonomic units. Finally, bioactivity screenings revealed that some isolates displayed high antibacterial activities, and genome mining uncovered a strong potential for the production of natural compounds.

Structural dynamics of the non-folded, translocation-competent secretome

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Sec-dependent secretory preproteins carry N-terminal signal peptides and are targeted post-translationally to the translocase complex SecYEG, while being maintained in non-folded states. Upon translocation, the signal peptide is cleaved and folding into mature native structures initiates (Tsirigotaki et al., 2017). Factors that delay folding in the cytosol are known but not the molecular basis of their effect. Both the type of signal peptide, the linkage to mature domain, the nature of the early mature domain and chaperones affect folding states (Sardis et al., 2017; Tsirigotaki et al., 2018). An additional feature is intrinsic to mature domains: they predominantly display slow folding and retain loose, long-lived intermediates. These properties emerge from residue composition, elevated disorder and reduced hydrophobicity (Tsirigotaki et al., 2018) and distinguish secreted proteins as a protein class, distinct from cytoplasmic folders.

To address what structural elements impact protein trafficking and folding, we analyse secretory proteins using hydrogen-deuterium exchange mass spectrometry (HDX-MS) and single molecule FRET (Förster Resonance Energy Transfer). With HDX-MS, the rigidity and overall structural dynamics of protein folding states are established by monitoring the exchange between amidic protons on the polypeptide's backbone with deuterium provided in the solution. smFRET analysis determines distance changes between fluorophore-labelled amino acid pairs in the sequence. As the polypeptide undergoes folding transitions, smFRET can determine folding intermediates, inter-conversion kinetics between states and lifetimes of states as well as chaperone binding.

We are focusing on several secretory proteins including maltose binding protein (MBP) and the prolyl cis-trans isomerases PpiA and PpiB as model proteins. HDX-MS on MBP has revealed distinct folding intermediates and these can be followed by smFRET. The native MBP structure folds in several minutes, whereas the presence of a signal peptide kinetically slows down folding. In contrast, the chaperone SecB alters the folding pathway by stabilizing more loose folding intermediates. Periplasmic PpiA and cytosolic PpiB are structural homologues, but show different HDX-MS-detectable folding kinetics. PpiA folds significantly slower than PpiB by retaining a conformationally loose intermediate state (Tsirigotaki et al., 2018). Extending this analysis, we are developing a smFRET pipeline to track the conformational dynamics of PpiA/B intermediates. Moreover, bioinformatics and structural analyses have led to the prediction of PpiB residues important for fast folding and these are being tested by site-specific mutations.

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Homoacetogenic CO_2 fixing activity and microbial community composition are shaped by pH and total sulfide concentration

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Carbon dioxide (CO_2) contained in waste gaseous streams, can be reduced into commodity chemicals through fermentation. Gas fermentations are one such application, where homoacetogenic bacteria are employed to convert CO_2 to acetate, using hydrogen (H_2) as electron donor, through the Wood-Lungdahl pathway [1]. A common contaminant of concern in most of these streams is hydrogen sulfide (H_2S), which is toxic, already at concentrations of a few ppm [2]. Pretreatment strategies for H_2S removal, already industrially applied, include absorption scrubbing and bio-oxidation to elemental sulfur [3]. Electrochemical treatment is one of the recently introduced approaches, where H_2S is oxidized to elemental sulfur [4] and hydrogen (H_2) is produced, which can boost the CO_2 conversion into soluble chemicals. The pretreatment steps will decrease the H_2S concentration , but a fraction of it will inevitably end up in the fermentation reactor where it can hamper bacterial activity. The extend of inhibition is expected to be determined by the operational pH of the fermentation, since this affects directly the sulfide speciation.

In this study, the main objective was to investigate the tolerance level of homoacetogenic bacteria to H_2S, in terms of inhibition of commodity chemicals bioproduction. Therefore, a series of toxicity experiments were conducted in serum flasks, inoculated with a mixed homoacetogenic microbial culture and a range of sulfide concentrations, from 0 up to 5 mM total sulfide. Sulfide toxicity was assessed at pH 5, 6 and 7, to make a distinction between H_2S and HS^- toxicity. Inhibition was evaluated based on acetate production by the microbial community. Increasing sulfide concentrations had an inhibitory effect to both acetate and biomass production. Maximum acetate production rates of 0.12, 0.09 and 0.04 mM h^{-1} were achieved in the controls without sulfide at pH 7, 6 and 5, respectively. The half maximal inhibitory concentration (IC_{50}) was 1.18, 1.15 and 1.08 mM [TDS] and 0.63, 0.76 and 0.75 mM [H_2S_{aq}] for pH 7, 6 and 5, respectively. At [TDS] above 4.29 mM, the acetate production and microbial growth were totally inhibited at all pHs tested. The 16S rRNA gene amplicon sequencing revealed major community composition dissimilarities that correlated with both pH and [TDS], indicating the importance of both the sulfide speciation and concentration for toxicity assessments.

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Enrichment and isolation of hydrogen-oxidizing microbiomes

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Knowledge about the enrichment of microbial communities growing on hydrogen, oxygen and carbon dioxide is scarce despite numerous studies on axenic cultures of hydrogen-oxidizing bacteria. In the present work, soil was used as an initial mixed culture for the enrichment of autotrophic hydrogen-oxidizing microbiome by means of a continuous supply of hydrogen, oxygen and carbon dioxide during 120 days with 5 media exchanges (5 transfers). The success of the enrichment was evaluated by monitoring ammonium consumption and biomass concentration during 5 transfers. The microbial community showed a clear distinction between the soil and transfers with a presence of numerous heterotrophs at final transfers in spite of hydrogen-oxidizing bacteria enrichment conditions using 16S rRNA amplicon sequencing. Moreover, isolation of some abundant autotrophs and heterotrophs was accomplished under heterotrophic and autotrophic conditions. *Hydrogenophaga electricum* was isolated and found to be one of the abundant species in most transfers. The findings obtained in this study clearly show a successful workflow to monitor the enrichment for hydrogen-oxidizing bacteria over a short period and retrieving some autotrophic and heterotrophic bacteria from the mixed community.

Circuitry rewiring directly couples competence to predation in the gut-dweller *Streptococcus salivarius*

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Small distortions in transcriptional networks might lead to drastic phenotypical changes, especially in cellular developmental programs such as competence for natural transformation. With a combination of deep sequencing methodes and phenotypical tests, we unearthed a pervasive circuitry rewiring for competence and predation interplay in commensal streptococci. Canonically, in model species of streptococci such as Streptococcus pneumoniae and Streptococcus mutans, the pheromone-based two-component system BlpRH is a central node that orchestrates the production of antimicrobial compounds (bacteriocins) and incorporates signal from the competence activation cascade. However, the human commensal Streptococcus salivarius does not contain a functional BlpRH pair and in this species, the competence signaling system ComRS directly couples bacteriocin production and competence commitment. This network shortcut might account for an optimal reaction against microbial competitors and could explain the high prevalence of S. salivarius in the human digestive tract. Moreover, the broad spectrum of bacteriocin activity against pathogenic bacteria showcases the commensal and genetically tractable *S. salivarius* species as a user-friendly model for natural transformation and bacterial predation or to mobilize bacteriocins inside the microflora.

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Discovery, Biosynthesis and Bioengineering of Antibiotic Natural Products from underexplored Gram-Negative Bacteria

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To date, the overwhelming majority of clinically-used antibiotics are derived from natural products produced by Gram-positive Actinobacteria. Bacterial pathogens are becoming increasingly resistant to these compounds, and there is an urgent need to discover novel antimicrobials to address the emerging health threat. In recent years, Gram-negative bacteria have become increasingly recognized as a rich and underexplored source of antimicrobial natural products with therapeutic potential. Enacyloxin IIa is a polyketide antibiotic that targets ribosomal elongation factor Tu.[1] It has been shown to have clinically-relevant activity against *Acinetobacter baumannii*, a problematic multidrug-resistant Gram-negative pathogen.[2] Despite its promising biological activity, enacyloxin IIa is unlikely to find direct clinical application, given the densely-packed array of similar and potentially labile functional groups in the antibiotic. We have idenfitifed the 80 kb enacyloxin gene cluster in the genome of *Burkholderia* species and proposed a pathway for its biosynthesis, involving assembly of the 25-carbon acyl chain by a modular polyketide synthase.[2] Detailed structural and biochemical analyses have provided insights into the final stages of enacyloxin assembly, including the unusual mechanism of chain release.[3,4] Using a variety of rational engineering approaches, including genome mining, mutasynthesis, gene deletion and feeding strategies, we have exploited this biosynthetic knowledge for the production of novel analogues with enhanced activity.[5]

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Urea nitrification and COD removal from synthetic urine by defined bacterial consortium for future space life support systems

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A life support system (LSS) in space aims at creating a safe and healthy environment for the astronauts. Nowadays LSS onboard ISS (International Space Station) provides water and oxygen by physico-chemical methods. Nevertheless, current LSS cannot provide food, which has to be delivered from Earth. Production of food can be achieved by utilizing regenerative life support systems (RLSS). MELiSSA (Micro-Ecological Life Support System Alternative) aims at producing food via vegetable crops and edible phototrophic bacteria. To produce this phototrophic biomass and to provide sustainability to the system, conversion of the resources in urine, a waste stream containing ~85% of the crew's nitrogen intake, into a nitrate and inorganic carbon substrate is needed. Conversion of urea to nitrate is very important, as nitrate is the most desirable nitrogen source for photoautotrophic organisms in future LSS. Despite the fact that urea and ammonium can also constitute a source of nitrogen for phototrophs growth on the Earth, these compounds are not advisable in closed space environments. Urea is very easily hydrolysed to ammonium, which is subsequently converted into toxic ammonia gas – compound not desirable in the space habitat's atmosphere. Human urine contains many types of organic components, which concentration equals approximately 10 g COD/L. The next challenge is to convert these COD components into carbon dioxide, which afterwards can be used by plants and cyanobacteria for the photosynthesis. All these processes are very efficient on Earth and they are conducted by complex microbial consortia, composed of many types of microorganisms, from which a lot are still unexplored and poorly understood. Nevertheless, the MELiSSA project focuses on bioprocesses with defined microbial communities. For systems like this it is easier to develop mathematical models, control the process and avoid occurrence of pathogenic strains onboard space habitat.

Human urine is a very complex matrix. To simplify the process for initial deeper understanding of the main reactions, it was decided to work with synthetic urine, which was developed to mimic real human urine composition.

For converting urea into nitrate and COD into carbon dioxide, it will be necessary to establish a bacterial consortium composed of strains allowing urea hydrolysis, ammonium and nitrite oxidation as well as COD oxidation into carbon dioxide. The main goal of the conducted research is to develop a synthetic microbial consortium able to perform these processes in the synthetic urine. Cultures of single heterotrophs were cultivated in synthetic urine and in real human urine to evaluate its urea conversion to nitrate and COD removal. Based on these results microbial consortia composed of AOB (ammonia oxidizing bacteria), NOB (nitrite oxidizing bacteria) and heterotrophic strains were established and tested in synthetic urine.

Linking microbial activity and the pore structure of a natural rock with X-ray Computed Tomography

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Bacteria can colonize porous rocks in natural outcrops or building stones. Many studies focused on the negative effect of the colonization, such as biodeterioration or they emphasised the beneficial effect like microbial induced calcium carbonate precipitation (MICP). Some aspects have however been neglected like the effect of biogenic gas production or the linkage between the pore structure of a rock.

Paracoccus denitrificans has been used as model organism together with two porous calcareous stones: Tabaire (Spain) with an open porosity and Savonnières (France) which has a complex pore structure. *Paracoccus denitrificans* is a denitrifier creating nitrogen gas out of nitrate which can induce at the same time calcium carbonate precipitation. The colonization potential has been roughly estimated with a flow cytometer. It counted the bacteria that were flushed through the stone samples. It the next phase *Paracoccus denitrificans* grew inside the porous rocks which were imaged several times using X-ray computed tomography. It revealed a significant amount of gas production inside the rocks and could link the gas bubble distribution to the pore structure. The open pore system of Tabaire contained less but larger bubbles, while the gas bubbles within Savonnières were more isolated from each other. MICP, the other potential reaction product has not been clearly identified. The results are very promising and show the potential of this visualization technique. The effect of the X-rays on the growth and activity of bacteria is a major concern of this technique. Exposure did not lead in another experiment to lower growth or a high direct mortality but more research is necessary to exclude this potential drawback.

First insight of microbial interactions between heterotrophic bacteria from river water.

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In rivers, heterotrophic bacteria play a key role in water 'self-purification' by their action of mineralization of organic matter and export of carbon to the atmosphere. 16S rRNA surveys have revealed that river waters contain hundreds to thousands of bacteria species. A majority of those are heterotrophs; yet their interplay in the degradation of organic matter remains uncharacterized. In this research project, we aimed to study the microbial interactions amongst a simplified model community of 20 heterotrophic bacterial strains isolated from the Zenne river (Belgium).

The growth rate of those strains in monocultures in R2B (batch experiments) and the growth rate of co-cultures in the same medium was assessed by spectrophotometry and flow cytometry. Growth rates in monocultures ranged from 0.06 to 0.95 h^{-1}, a wide range of values which remain however within the range of those mentioned in the literature for freshwater bacteria.

To infer the type of interaction between co-cultured strains, we compared the sum of the growth rate of the two monocultures with the growth rate of co-culture according to Freilich *et al.*, 2011 [1]. Such calculations revealed that all interactions were competitive (indeed moderately competitive), except one co-culture with no interaction and one cooperative interaction. The prevalence of competitive interactions could easily be explained by our experimental set-up: strains were cultured in R2B medium which is richer in nutrients than their natural habitat, we operated in batches so there was no fresh input of nutrients all over the incubation period, and the environment was homogeneous. Yet, we plan to check by alternative methods the inference of interactions based on growth rates of monocultures and co-cultures.

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Monitoring protein secretion in *Streptomyces* using fluorescent proteins

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Fluorescent proteins are a major cell biology tool to analyze protein sub-cellular topology. Here we have applied this technology to study protein secretion in the Gram-positive bacterium *Streptomyces lividans* TK24, a widely-used host for heterologous protein secretion biotechnology. Green and monomeric red fluorescent proteins were fused behind Sec (SP^{Sec}) or Tat (SP^{Tat}) signal peptides to direct them through the respective export pathway. Significant secretion of fluorescent eGFP and mRFP was observed exclusively through the Tat and Sec pathways, respectively. Plasmid over-expression was compared to a chromosomally integrated *sp^{Sec}-mRFP* gene to allow monitoring secretion under high and low level synthesis in various media. Fluorimetric detection of SP^{Sec}-mRFP recorded folded states, while immuno-staining detected even non-folded topological intermediates. Secretion of SP^{Sec}-mRFP is unexpectedly complex, is regulated independently of cell growth phase and is influenced by the growth regime. At low level synthesis, highly efficient secretion occurs until it is turned off and secretory preforms accumulate. At high level synthesis, the secretory pathway overflows and proteins are driven to folding and subsequent degradation. High-level synthesis of heterologous secretory proteins, whether secretion competent or not, has a drastic effect on the endogenous secretome, depending on their secretion efficiency. These findings lay the foundations of dissecting how protein targeting and secretion are regulated by the interplay between the metabolome, secretion factors and stress responses in the *S. lividans* model.

Keywords:

eGFP, mRFP, protein secretion, signal peptide, *Streptomyces lividans*, protein secretion biotechnology

Isolation, characterization and determination of biotechnological potential of oil degrading bacteria from Algerian centre coast

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The Algerian coastline is exposed to several types of pollutions, including hydrocarbons (HC). The aim of this work was to isolate oil-degrading bacteria and to explore the intrinsic bioremediation potential of one of its contaminated harbour.

A collection of 119 strains, capable to grow on mineral medium supplemented with HC, were obtained from polluted sediment and seawater collected from Sidi Fredj harbour (Algiers). 23 strains were selected for further studies. Sequencing of the 16S rRNA gene showed that most isolates belong to genera of hydrocarbonoclastic bacteria (*Alcanivorax*), generalist hydrocarbons degraders (*Marinobacter, Pseudomonas, Gordonia, Halomonas, Erythrobacter and Brevibacterium*) and other bacteria not known as hydrocarbon degraders (*Xanthomarina*) but were able to degrade HC. Strains related to *Marinobacter* and *Alcanivorax* were dominant and the most effective in degrading crude oil. Screening of catabolic genes *alkB* and *xylA*, revealed the presence of *alkB* gene in several bacterial strains, one isolate harboured both catabolic genes while some isolates carried none of the studied genes. However, they grew in the presence of crude oil implying the existence of other biodegradation pathways.

The Algerian collection of hydrocarbon degrading bacteria contains potential candidates for bioremediation purposes and can be useful for biotechnological applications.

Keywords: Bioremediation, hydrocarbon degradation, oil degrading bacteria, catabolic genes, biosurfactant

Urban endotoxins: abundance and inflammatory capacity in polluted air

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Endotoxins are components of Gram-negative bacteria with inherently high pro-inflammatory potential. In an urban environment, airborne endotoxins associate with pollutants such as particulate matter (PM) and may play an important role in the resulting adverse health effects. In this study, we collected air samples in different land-use classes (urban traffic, urban green, industrial) throughout the city of Antwerp. We subsequently measured endotoxin concentration (recombinant Factor C (rFC) assay), human host recognition of endotoxin *in vitro* (HEK Blue-TLR4 NF-kB reporter cell ine), pro-inflammatory response markers (IL-8, IL-1β, TNFα gene expression in U937 cells) of air samples, particle count, and transition metal composition (ICP-MS). Endotoxin concentrations from 87 samples distributed throughout the city ranged from 0.45 to 93.71 EU m^{-3}, with a geometric mean of 4.49 EU m^{-3}. We found that this endotoxin concentration assay did not correlate strongly with the recognition of endotoxins by human Toll-like receptor 4 (hTLR4) in human cell lines, which was significantly affected by land-use class. Here, traffic locations were found to be significantly higher in bioactive endotoxin than the industrial and green locations. We subsequently turned our attention to how host recognition of endotoxin through TLR4 may be affected by its association with other PM components such as transition metals. The effect of nickel and cobalt – previously reported to independently activate hTLR4– was found to be negligible in comparison to that of iron. Here, the addition of iron as a factor significantly improved the regression model between the two endotoxin assays, explaining 77% of the variation of the TLR4 stimulation and excluding the significant effect of land-use class. Moreover, the effect of iron proved to be more than a correlation, since dosing LPS with Fe^{2+} led to an increase up to 64% in TLR4 stimulation, while Fe^{2+} without LPS was unable to stimulate a response. This study shows that endotoxin quantification assays (such as the rFC assay) may not always correspond to human biological recognition of endotoxin in urban PM, while its toxicity can be synergistically influenced by the associated PM composition.

Directed evolution of *Bacillus cereus* endospores for improved heat resistance

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Bacterial endospores are the most robust lifeform on earth, and eradicating spores of pathogenic bacteria in food and in the food processing environment is sometimes problematic because of their recalcitrance. Most studies about spore inactivation have so far focused on efficacy of treatments and spore resistance mechanisms, however information on the selective pressures of sporicidal treatments are scarce. Here we examined the evolutionary ability of *B. weihenstephanensis*, a psychrotrophic member of the *Bacillus cereus sensu lato* group, to yield endospores with increased heat resistance. Three independent lineages of *B. weihenstephanensis* LMG 18989 endospores were iteratively subjected to sporicidal heat stress (started from 93°C for 4 minutes, normally resulting in 2-3 log inactivation of wild type spores) with intermittent germination and sporulation steps. Interestingly, each of the three heat stressed lineages developed significant heat resistance already after 2nd-3rd stress cycles, while control lineages that were cycled without heat stress did not. Purified single clones from the stressed lineages had between 2,500 and 12,000-fold higher survival rate than the parental strain upon heating at 97°C for 5 minutes, confirming that they had developed increased heat resistance. Phenotypic characterization and whole genome sequencing of these clones are in progress to further characterize the exact resistance mechanism. This study underscores that endospore heat resistance is a readily evolvable trait.

Dynamic genetic adaptation in the bacterium *Cupriavidus metallidurans* in response to uranium

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The bacterium *Cupriavidus metallidurans* is a well-known model organism for metal resistance and is mostly isolated from industrial sites linked to mining, metallurgic and chemical industries. The interaction of *C. metallidurans* with uranium (U^{238}) and americium (Am^{241}) has been demonstrated, however, neither the genes or proteins involved nor the precise mechanism is known.

Exposure of *C. metallidurans* NA4 to high concentrations of $UO_2(NO_3)_2$ showed that NA4 is able to rapidly adapt to toxic uranium concentrations. The spontaneous mutant NA4U was isolated and resists up to 1000 µM. In contrast, the minimal inhibitory concentration of $UO_2(NO_3)_2$ for the parental NA4 strain is 125 µM. Whole-genome expression profiling in non-selective growth conditions showed that in NA4U 541 genes were upregulated and 328 downregulated compared with the parental strain. Next to canonical resistance mechanisms, genes coding for uncharacterized small periplasmic proteins were upregulated as well. The two-component system *czcR2S2* is under investigation as well since whole-genome resequencing of NA4U showed a 900 bp deletion in the sensor histidine kinase CzcS2, probably causing the constitutive over-expression of the response regulator (*czcR2*). Deletion of *czcR2S2* from NA4U showed clear loss of resistance at 500 µM $UO_2(NO_3)_2$, serving as evidence for the central role of CzcR2 in the uranium resistance phenotype. Furthermore, growth of NA4U in presence of 340 µM $UO_2(NO_3)_2$ indicated a clear increase of uranium in the cell pellet after 24 hours, with a simultaneous decrease of uranium in the supernatant. Contrary, cell pellets of autoclaved cells showed no increase in uranium. These results serve as preliminary evidence that NA4U is using an active mechanism to retain uranium from the medium since the amount of uranium/cell increased as well. However, further tests and repetitions to provide more evidence are needed. These observations are strikingly similar to those observed in a silver-adapted *C. metallidurans* strain.

This study underscores the rapid evolution of *C. metallidurans* towards significantly increased uranium resistance and is a first step in unravelling its cellular response to uranium. Investigating the uranium resistance mechanisms of *C. metallidurans* NA4U can provide further insights in the microbial impact on the long-term behaviour of radionuclides.

Highest diversity of cyanobacteria on granite substrates in the Sør Rondane Mountains

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Abstract: In the Sør Rondane Mountains (Dronning Maud Land, East Antarctica), cyanobacteria are mainly present in sheltered spots in rocky areas, supporting the importance of micro-topographic and -climatic conditions. Biofilms, crusts and gravels on granite and gneiss substrates were sampled in 2009-2010 near the Belgian Princess Elisabeth Station and their morphological (126 samples) and molecular diversity (26 samples) was assessed. A DNA extraction protocol was designed for the taxa with large polysaccharidic sheaths. Based on microscopy, crusts were the richest samples followed by gravels. The most diverse communities were found on the granites. Based on DGGE of 16S rRNA gene, 28 OTUs shared at least 97.5% of 16S rRNA similarity. OTUs' richness varied between 1 and 5 per sample. A comparison of morphological and DGGE analyses showed that for most samples, the number of morphotypes was higher than the number of OTUs. However, both methods were congruent in defining the richest sites. The most frequently observed OTU was affiliated to *Phormidium/Microcoleus* sp. No OTU was common to all the 10 sites. The higher diversity on granite substrates (mainly big boulders) could be explained by higher stability of the underlaying rock and its ability to keep water on the surface, thus creating favorable conditions for development of organisms. Further insights will be given by the analysis of more samples with High-Throughput amplicon sequencing.

Following the impact of metals on river sediments: metals as a community manager

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Metal contamination of soil and sediments is a serious environmental issue as metals cannot be degraded. Despite elevated metal concentrations, river sedimentary microbial communities near the MetalEurop foundry (Northern France) display an unexpected high diversity in comparison with an upstream control site (Férin). Recent methodological improvements of 16S rRNA taxonomy profiling has enhanced analytical accuracy and revealed that metals act as drivers of the microbial community structure. In the present study, a follow-up of the evolution of sediment microbial communities sampled in Férin sediments was performed in microcosms with a periodic renewal of the supernatant water. Experimental microcosms were progressively exposed to a mixture of metals to finally reach concentrations observed in MetalEurop: Cd (38.1 mg/kg), Cu (100.0 mg/kg), Pb (913.8 mg/kg) and Zn (3218.5 mg/kg). Microcosms were followed using 16S rRNA gene Illumina sequencing. Functional insights on metal resistance were obtained by quantitative PCR targeting *czc*A and *pbr*A genes coding for metal efflux pumps. The broad host range incP plasmid content was also followed by quantitative PCR. Interestingly, taxonomical analyses revealed a higher specific richness and equitability in metal-contaminated microcosms. This increase can be explained by metals acting as a community-manager together with community coalescence (i.e., the gathering of two different microbial communities). Quantitative PCR analysis coupled with taxonomic evolution suggest a step by step adaptation through the selection of different metal-resistance mechanisms.

Assessing the microbiological diversity in the cooling waters of a nuclear research reactor

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The BR2 nuclear research reactor consists of different watery environments, one of which is an open basin surrounding the reactor vessel. The water in this basin has a shielding effect on the radiation originating in the reactor core, where nuclear fission takes place during reactor operation. Remarkably, despite the ultra-high purity of the water due to constant filtering and deionization, combined with the high radioactivity exposure, microbial growth in these environments is not fully prevented. Indeed, several microbes appear to be able to survive and thrive in such conditions. Microorganisms identified in those environments thus provide a unique opportunity to acquire new insights into survival strategies and radiation-resistance mechanisms.

The objective of this work is to explore the bacterial communities present in the basin water of the BR2 nuclear research reactor. In order to accomplish this, the bacterial population was followed up over time during and outside reactor operation to monitor its dynamics in this unique, never-before-studied environment.

For the characterization and the follow-up of the bacterial communities, a 16S rRNA amplicon sequencing approach was adopted. Results from two long-term follow-up experiments highlighted a clear shift in the bacterial community profile during and outside reactor operation. Interestingly, the profiles for both experiments appeared to be quite similar, notwithstanding the fact that the two sampling campaigns were separated by a one-year interval. This indicates that the system is very robust.

During reactor operation, the bacterial community is mostly dominated by two OTUs that were taxonomically assigned to an unclassified Gammaproteobacterium and *Pelomonas*, respectively. During this phase, either one or the other becomes prevalent, or both are equally abundant. When the reactor goes into shutdown, the community clearly shifts to become dominated by an OTU assigned to *Methylobacterium*. This interesting finding can be explained by the change in physico-chemical parameters like flow rate, temperature and most importantly radiation that occurs when the reactor transitions from one phase to the other. In addition, exposure to radiation also causes the bacterial population to steeply decrease in number, before it can slowly recover during reactor shutdown.

To conclude, we have been able to shed some light on this unique system for the first time and thoroughly explored its dynamics. In a next step, we will further investigate the radiation resistance potential of some interesting isolated strains.

Niche specificity of lactobacilli in fermented carrot juice as a model ecosystem

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The genus *Lactobacillus,* which contains more than 200 different species, shows a remarkable large diversity compared to the diversity within other bacterial genera. Lactobacilli can be found in a wide variety of niches including: different human body sites, plants, and animal products (e.g. milk, cheese, yoghurt and meat) and are important drivers in many microbial ecosystems. This large diversity and different niche specificities is something that intrigues us. Therefore, we will explore the niche specificity of lactobacilli by introducing strains isolated from different origins (autochthonous vs allochthonous) in a model ecosystem and assess their impact on the microbial community. Here, we propose the use of fermented foods as tractable model ecosystems, since these fermentations have been shown to be repeatable, manipulatable and can be easily followed over short-time periods. Previous work in our lab clearly showed the opportunity of carrot fermentation as a robust man-made model ecosystem with well-defined microbial dynamics, revealed by our novel RNA-based 16S amplicon sequencing approach. Current results indicate that autochthonous LAB isolates have a superior capacity to ferment carrots compared to human isolates. E.g. the autochthonous *Leuconostoc* strain showed the fastest decline of pH, reaching pH <5 after the first day. After 30 days of fermentation, the juices with autochthonous LAB as starter culture, also contained a higher relative abundance of *Lactobacillus* species compared to juices with allochthonous LAB from other niches as starter culture.

Air pollution and plant-microbe interactions in the phyllosphere

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The surface of plant leaves, also termed the phyllosphere, is a unique habitat for microbes. The bacterial composition of the phyllosphere seems to depend on the plant host species, leaf characteristics, season, climate, and location of the plant host. In this study, we investigated the effect of an urban environment and air pollution on the composition of phyllosphere communities. Leaves were sampled from 18 ivy plants (*Hedera sp.*) and 56 London plane trees (*Platanus* × *acerifolia*) in the city of Antwerp and its surroundings. The bacterial community composition was determined using 16S rRNA gene sequencing on the Illumina MiSeq platform. Leaf biomagnetic analyses were used to estimate exposure to particulate matter, a major component of air pollution. We found that many of the dominant taxa of the ivy phyllosphere communities were strongly correlated with the leaf biomagnetic signal. Interestingly, some of these taxa indicate differences in the available substrates on ivy leaves of the city compared to a less urbanized environment. However, dominant taxa of London plane tree communities were not correlated with the biomagnetic signal - a proxy for leaf deposited particulate matter –, although they showed a strong correlation with car traffic intensity. This further suggests that gaseous traffic-related pollutants affect the phyllosphere bacteria. Furthermore, leaf characteristics, such as chlorophyll content and leaf wettability were linked with the plane tree phyllosphere composition. Although interesting associations were found, the mechanisms by which land use, car traffic intensity and air pollutants affect the phyllosphere community composition remain to be explored.

Rapid purification and characterization of recombinant proteins and antibodies: Capturem high-capacity membranes

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Recombinant protein production is immensely important in many research settings, including academic research institutions, biopharmaceutical organizations, and enzyme and agricultural industries. Fusion tags are widely used to improve yields and enable purification and characterization of protein structure and function. Polyhistidine tags, which incorporate 6–10 histidines at either terminus of the target proteins, are the most popular tag used for purification. The affinity of the histidines to immobilized metal ions such as Co2+ and Ni2+ is utilized to selectively bind the tagged construct to the matrix, while washing away unwanted materials, before eluting the target with low-pH or imidazole-containing buffers. Typical purification methods using immobilized metal affinity chromatography (IMAC) columns take several hours to complete due to long column equilibration/binding times and slow diffusion of large macromolecules through the resin bed. The long times increase the risk of proteolytic degradation and activity loss due to unfolding or denaturation. Membrane-based affinity systems have rapid flow-induced mass transport and short residence times; however, they have been plagued with low capacity due to small internal surface areas. Here, we describe a novel, nylon-membrane-based IMAC system with a chemically enhanced surface area of the pores that allow protein binding capacities comparable to, or better than, resins at 75 mg or more per cm3 of membrane. Unlike traditional resin-based systems, the entire purification process—from loading the lysate to eluting pure protein—can be completed at room temperature in less than five minutes. We have assembled these membranes into spin columns and filtration devices and demonstrated their ability to purify his-tagged proteins produced in bacterial and mammalian cells. The millisecond residence time of the proteins on the membrane during binding minimizes the possibility of degradation. These membranes function perfectly in the presence of additives such as ethylenediaminetetraacetic acid (EDTA), reducing agents such as dithiothreitol (DTT), and under denaturing conditions (in the presence of urea and guanidium hydrochloride). We have extended the high-capacity membrane technology to immobilize Protein A and G, enabling extremely fast purification of antibodies from various matrices based on the affinity of these proteins for the fragment crystallizable region (Fc) region of antibodies. Antibody purification can be accomplished in less than 10 minutes, with capacities of up to 75 mg/ml or more, far exceeding the capacity of resin-based columns. More recently, we have immobilized trypsin and pepsin enzymes on these membranes to carry out proteolysis of proteins for their characterization, identification, and quantitation through mass spectrometry analysis. In contrast to the long incubation period (6–24 hours) of conventional in-solution digestions, the proteolytic membranes generate peptides suitable for downstream analysis, with the same or improved sequence coverage, in less than a minute, for downstream analysis. Additionally, we are expanding the membrane technology with immobilized streptavidin suitable for enrichment of target proteins, antibodies, and oligos. These novel membrane-based spinnable affinity columns and filtration devices will be useful for purifying a variety of recombinant proteins and antibodies and their proteomics characterization in academic and industrial settings.

ABSTRACTS POSTERS

SECTION C: MEDICAL AND VETERINARY MICROBIOLOGY

A Gammaherpesvirus Affects Type 2 Innate Lymphoid Cells in the Context of HDM-Induced Asthma

Authors

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Persistent viruses, such as gammaherpesviruses, profoundly imprint the immune system of their hosts. Accordingly, infection with murid gammaherpesvirus 4 (MuHV-4) inhibits the development of airway allergy following the instillation of house dust mite extracts (HDM) in mice. Group 2 innate lymphoid cells (ILC2s) play a major role in the initiation, maintenance and memory of type 2 immune responses. This work aims to investigate the impact of MuHV-4 infection on pulmonary ILC2s. Our results showed that MuHV-4 infection modifies functional properties of ILC2s as early as 8 days post-infection. Specifically, the production of type 2 cytokines such as IL-5 and IL-13 by ILC2s was decreased after HDM-sensitization or allergic challenge. This reduced cytokine production was associated with the decreased expression of the canonical Th2 transcription factor GATA-3. Although not exhibiting any characteristic of plasticity towards an ILC1 phenotype, these ILC2s from MuHV-4 infected mice showed a lower expression of the PD-1 and KLRG1 activation markers. Accordingly, MuHV-4 infection impaired the recruitment of some described "inflammatory" ILC2s without affecting the multiplication of the "resident" ILC2s. Overall, these results show that MuHV-4 infection significantly and sustainably affects the lung ILC2s population and that this may have a determining role in the subsequent development of immune responses against respiratory allergens.

The effect of fosmidomycin prodrugs against *Acinetobacter baumannii* and *Burkholderia cenocepacia*

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With increasing antibiotic resistance, infections by many pathogens are becoming more difficult to treat. These include *Burkholderia cenocepacia*, which is a common pathogen in cystic fibrosis, and *Acinetobacter baumannii,* which is a common pathogen responsible for various types of nosocomial infections. As such it is necessary to develop new drugs to either treat infections or to potentiate existing drugs. Fosmidomycin was originally developed as an antimalarial drug that targets the non-mevalonate pathway for isoprenoid synthesis. Isoprenoids can be produced through the mevalonate and/or the non-mevalonate pathway and multiple bacterial pathogens exclusively use the non-mevalonate pathway for isoprenoid synthesis; as such these could potentially be susceptible to fosmidomycin and its (prodrug) derivatives. *A. baumannii* and *B. cenocepacia* are two such pathogens that use the non-mevalonate pathway.

Activity of the prodrugs was evaluated by determination of the minimal inhibitory concentration (MIC) against strains of *A. baumannii* and *B. cenocepacia*. The ability of the prodrugs to potentiate antibiotics was evaluated using checkerboard assays to determine the fractional inhibitory concentration index (FIC index) of the combinations.

We found a MIC of 1 µg/mL for the best performing prodrug (CC271) against *A. baumannii* LMG10520. Two more prodrugs demonstrated a high activity with a MIC of 2 µg/mL for CC366 and 8 µg/mL for CC341. These prodrugs were also active against multiple strains of *A. baumannii* (AB5075, LMG989, LMG10531, NCTC13423, R-67512, and RUH134) The prodrugs were less active against *B. cenocepacia* K56-2, with a MIC of 32 µg/mL for CC271 and a MIC of >64 µg/mL for CC341 and CC366. However combinations with aztreonam and ceftazidime resulted in a synergistic effect between the prodrugs and the antibiotics. CC271 and CC341 in combination with aztreonam resulted in a FIC index of 0.16 and 0.19 respectively, whereas a combination with ceftazidime resulted in a FIC index of 0.31 for both prodrugs.

The results demonstrate the potential of these prodrugs for treatment of *A. baumannii* and a potentiating effect for treatments of *B. cenocepacia*. We are currently investigating the effectiveness of these compounds against biofilms of these pathogens.

Capsular serovars of virulent *Capnocytophaga canimorsus* are shared by the closely related species *C. canis* and *C. cynodegmi*.

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Capnocytophaga canimorsus, *Capnocytophaga cynodegmi and Capnocytophaga canis* are gram-negative bacteria living as commensals in the mouth of dogs and cats. While *C. cynodegmi* and *C. canis* can rarely cause mild local infections in humans that have been in contact with their animal hosts, *C. canimorsus* can cause rare but life threatening generalized infections.

Recently, we reported the discovery of a capsular polysaccharide (CPS) at the surface of *C. canimorsus* that is implicated in resistance to the innate immune system of the host.

We developed the first *C. canimorsus* capsular serotyping scheme and identified nine different serovars (A to I). This serotyping scheme allowed typing of 25/25 *C. canimorsus* human isolates but only 18/52 dog isolates, indicating that the repertoire of CPS in the species is vast. However, while only three serovars (A, B, and C) covered 88% of the human isolates tested (22/25), they covered only 7.7% of the dog isolates (4/52). Serovars A, B, and C were found more often among human isolates than among dog isolates, with no geographical bias, implying that strains endowed with these three CPS types are more virulent for humans than others.

Here, we serotyped 112 strains of *Capnocytophaga* spp. from the Culture Collection of the University of Gothenburg (CCUG) isolated from human infections. The *C. canimorsus* strains (86 of 96, 89.6%) belonged to serovars A, B, or C with relative frequencies of approximately 30% for each serovar. The high prevalence of the A, B, and C serovars in strains isolated from humans, compared to the previously described low prevalence of these serovars among dog isolates, confirms that these three serovars are more virulent to humans than other serovars and suggests that the low incidence of disease may be linked to the low prevalence of the A, B, and C serovars in dogs.

We then serotyped six human-isolated *C. canis* and ten *C. cynodegmi* strains and, surprisingly, found one *C. canis* and three *C. cynodegmi* strains to be of capsular serovar B. This observation prompted us to test 34 dog-isolated *C. canis* and 16 dog-isolated *C. cynodegmi* strains. We found four *C. canis* strains belonging to serovar A and one to serovar F. In contrast, no dog-isolated *C. cynodegmi* could be typed with the available antisera. This work demonstrates that virulence-associated CPS (A, B, and C) are not specific to the *C. canimorsus* species.

The effect of airway epithelial cells on the susceptibility of cystic fibrosis clinical isolates of *P. aeruginosa*

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Pseudomonas aeruginosa is considered as one of the most important pathogens in the pathology of cystic fibrosis (CF), as 80% of the adult population is infected with this pathogen which is strongly correlated with a higher morbidity and mortality. In the CF lungs, *P. aeruginosa* forms biofilms, which are highly tolerant and resistant to antibiotics and result in chronic infections. Thus, providing an effective treatment for these infections is an important aspect in improving the quality of life and prolonging the life expectancy of CF patients.

The use of antibiotics is generally recommended for the treatment of *P. aeruginosa* chronic lung infections, but does not always lead to clinical improvements. Indeed, a main gap exists between the efficacy of antibiotics *in vitro* and in CF patients. A reason for this discrepancy might be that environmental factors of the CF lung are not considered when evaluating antibiotic efficacy. This includes host cells, such as lung epithelial cells, which have been previously shown to modulate the efficacy of antibiotics. However, it remains unknown whether this effect varies depending on the patient from whom the lung epithelial cells were derived (carrying a different genetic background).

To address this research question, a method was developed to co-culture in vivo-like three-dimensional (3-D) models of different airway epithelial cell lines (including the CF cell lines CFBE and IB3 – derived from patients) with clinical CF isolates of *P. aeruginosa* (including AMT0023-34) to perform biofilm eradication studies without affecting host cell viability (as determined by LDH assay). The efficacy of antibiotics to eradicate biofilms was determined in the presence or absence of the different epithelial cells, by treating host-associated biofilms with a 2-fold dilution series of tobramycin in a concentration range that allows 90% reduction of the biofilm. All experiments were performed in microaerophilic conditions (3% O_2, 5% CO_2) relevant for the CF lung. Quantification of biofilms was done by plating.

Preliminary results for the strain AMT0023-34 show that the influence of airway epithelial cells on antibiotic susceptibility of *P. aeruginosa* differs depending on the source of airway epithelial cells, with some epithelial cells improving the efficacy of this antibiotic while others affect its efficacy. This suggests that the influence of airway epithelial cells on antibiotic efficacy might vary for different patients.

Uncovering actors and understanding mechanism of enterococcal β-lactam resistance

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During the last decade, enterococci emerged as one of the main cause of infection in hospital intensive care units, mostly, as result of their ability to resist to commonly used antibiotics.

Penicillin binding proteins (PBPs) are the β-lactams target. *Enterococcus hirae*, our model organism, presents 7 PBPs including low-affinity PBP5, responsible of β-lactams resistance. In highly resistant isolates, PBP5 is overexpressed, however, the mechanism leading to overexpression remains unknown. *pbp5* is included in an operon downstream *ftsW* and *psr*. Psr, supposed to be PBP5 synthesis repressor, is actually a LytR-CpsA-Psr family protein functionally related with bacterial cell wall polymers. Its specific role is still unknown, however, recent results suggest that Psr catalyses the transfer of a rhamnose-containing polysaccharide from a lipidic transporter on *E. hirae* peptidoglycan.

Penicillin-resistant mutants were selected by successive passages on increasing antibiotic concentration in order to study the mechanism underlying the resistant phenotype.

In this study, we were able to show that PBP5 overexpression is not due to mutations in the operon. By different techniques like genome-wide sequencing, RNA-seq and peptidoglycan analysis we were also able to identify and to test new actors involved in resistance.

Isoniazid bactericidal activity involves energetic perturbation

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Multi-drug resistance and recalcitrant mycobacterial persistence to conventional anti-TB drugs are two of the main obstacles for successful treatment and global control of tuberculosis. Stress conditions such as hypoxia and starvation are known to generate persistent population tolerant to isoniazid (INH), a key first-line bactericidal anti-tuberculosis drug currently known to inhibit mycolic acid synthesis. Here, we report that INH exposure rapidly increased *M. bovis* BCG cellular ATP levels, oxygen consumption and ROS generation. The INH-triggered ATP increase and bactericidal activity were strongly compromised by Q203 and bedaquiline inhibiting mycobacterial cytochrome bc_1 and F_0F_1 ATP synthase, respectively. Furthermore, the antioxidant N-acetylcysteine (NAC) but not 4-hydroxy-TEMPO (TEMPOL) abrogated the INH-triggered ATP increase and killing. These results reveal a link between the energetic perturbation and INH's killing. Furthermore, the INH-induced energetic perturbation was not abrogated by chemical inhibition of NADH dehydrogenases (NDHs) and succinate dehydrogenases (SDHs) but could be linked to membrane potential dissipation via the F_0F_1 ATP synthase. Importantly, a cytochrome *bd* oxidase inhibitor abolished the persister formation after INH exposure, suggesting that the respiratory reprogramming to the cytochrome *bd* oxidase contributes to INH tolerance. This study unveils a novel killing mechanism of INH involving the electron transport chain (ETC) perturbance and pinpoints the participation of the cytochrome *bd* oxidase in INH tolerance under stress conditions.

Key words: Isoniazid; Electron transport chain; Energetics; Cytochrome *bd* oxidase; F_0F_1 ATP synthase; Persister

Development of a proficiency testing for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by molecular techniques in human samples following ISO17043 standard.

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In the medical diagnosis of infectious diseases, the molecular techniques are now widely used. A reimbursement of these assays by the social security is foreseen. Since 2008, Sciensano (formerly WIV-ISP) was in charge of the organization of the proficiency testing (PT) for the Belgian clinical laboratories performing these assays in accordance to the official nomenclature. The participation to such a proficiency testing is mandatory for the Belgian laboratories to obtain their license and to benefit of the reimbursement of their assays. In 2010, a specific ISO standard, ISO17043, was created to explain how to organize the proficiency testing. Among the specifications, to guaranty the homogeneity and the stability of the proficiency testing samples is a major concern.

C. trachomatis and *N. gonorrhoeae* are two bacteria responsible of sexually transmitted infections. C. trachomatis is one of the most common human pathogens causing hyperendemic blinding trachoma. Three serotypes (L1, L2 and L3) caused lymphogronuloma venereum (LGV). C. trachomatis is the most common sexually transmitted bacterial pathogen causing genital tract infections and sporadic cases of conjunctivitis. C. trachomatis is a major cause (with *N. gonorrhoeae*) of urethritis in men. In women, C. trachomatis causes cervititis and acute salpingitis. The number of cases in Belgium increased in the last years from 2084 cases in 2005 to 6063 cases in 2015.

N. gonorrhoeae is responsible in men for acute urethritis with classical symptoms of burning during urination but asymptomatic infections are also quite frequent (up to 50%). If untreated, men may develop epididymitis, prostatitis and urethral stricture. In women, the primary site of infection is usually endocervix; symptomatic uncomplicated infections are characterized by vaginal discharge, dysuria and an erythematous, friable cervical os. The number of cases in Belgium increased from 448 in 2005 to 1339 in 2015.

To develop proficiency testing for both pathogens, cultures of the bacteria were performed. Then,

Urines and transport medium (M4RT) were spiked with dilutions of the bacterial cultures. The batch solutions were then divided into aliquots. A panel of samples consisted of either urines or transport medium spiked with several concentrations of each pathogen or unspiked (negative samples). Before to be sent to the participating laboratories, the panel was validated by the National Reference Center (ITM, Antwerpen, Belgium). The results of the NRC were considered as the target values for the PT. The number of participants in 2017 was 77. The laboratories returned their results to us indicating the presence or absence of *N. gonorrhoeae* and C. trachomatis in the different samples of the panel and also the used detection method. The individual results were then compared to the expected results. For example, in 2017, ther percentage of correct answers was 97.5% for both pathogens. For C. trachomatis in urines, 3 false negative and false positive results were encoded, for C. trachomatis in swaps (transport medium), 5 false positive results were encoded; for *N. gonorrhoeae* in urines, 1 false positive and 5 false negative were encoded and for *N. gonorroheae* in swaps, 4 false positive results were reported.

Therefore, an external quality assessment of the molecular diagnosis of the presence of C. trachomatis and N. gonorrhoeae in human samples remains important allowing the laboratories to evaluate and improve their methods.

SEASONAL EVOLUTION OF THE DIARRHOEA WITH ROTAVIRUS 2009 TO 2016 CASE OF THE MONITORING SENTINEL IN CH KINGASANI II/RDC

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Introduction In Democratic Republic of Congo, the monitoring of Rotavirus is carried out only in some medical structures from Kinshasa and Lubumbashi. There for, few data only exist on the epidemiology and the biology of Rotavirus.

Thus, no study has been conducted in the western sector of Kinshasa on the predominance and the molecular forms or the epidemiology of Rotavirus.

Yet, we think that the data resulting from this study will contribute to the establishment of the vaccine against this infant danger which does not cease plunging into mourning the Congolese house hold sin order to have a good care of children from 0 to 59 months suffering from this infection.

General Objective Determine the prevalence and molecular characteristics of the Rotavirus strain detected in the children admitted to the Hospital of Kingasani 2, in Kinshasa from 2009 to 2016 for treatment of acute diarrhea.

Methodology To achieve our purpose, we proceeded by collecting and testing stools samples from children of than 0 to 59 months hospitalized, presenting symptoms of then on-bloody diarrheas during at least 7 days.

Result We have noticed that there were more children suffering from the gastroenteritis during the period of 2015-2016 with 251 cases, 149 positive cases, or (59.3%) and 102 cases due to other diarrheic entéropathogènes or (40,7%), compared to each annual interval between 2009 and 2014 of our previous study. July had more cases with a frequency of 189 cases (15.9%), August with 165 cases (13.8%) ; June 149 cases (12.5%) May 135 cases (11.3%). Taking into account the number of the children suffering from the gastroenteritis attending the hospitals, we notice that frequencies according those ears and months are different and this could be due to the various climatic seasons. It is shown that during droughts and cold seasons which generally over May, June, July, August and September, children of 0 to 59 months are vulnerable to many epidemics such as gastro enteritis from diarrheas of various origins.

Conclusion This study was conducted in order to protect and prevent the danger swhich the human lives in general and children of 0 to 59 months in particular on the Rotavirus infection. We noted some risk factors to the exposure of Rotavirus, the therapy in case of Rotavirus infection, prevalence, the seasonal variations of this prevalence and Rotavirus strains circulating in the children admitted for the gastro enteritis. July with 189 cases (15.9%) had more children, August 165 cases (13.8%).

The Rotavirus detection was carried out during all they ear wither markable peaks around the months of the dry season (May, June, July, August and around September).

The season affects the prevalence of Rotavirus because there is a large number of cases of the diarrheas and the positive cases of Rotavirus during these months which are generally dry and cold in the town of Kinshasa. Therefore, it is very important to immunize the children of this age by the administration of a vaccine which would be able to through away the burd en of the diarrheal disease.

TEMPORARY FOLLOW-UP OF THE PREVALENCE OF GASTRO ENTERTAINMENT IN ROTAVIRUS

FREQUENCY OF CASES FROM 2009 TO 2017 AT KINGASANI HOSPITAL CENTER

D. Disengomoka G. Kitambala, J. Emonmey , D. Masungi

Foreword Rotavirus diarrhea is one of the significant causes of children's hospitalisation, it graws family budget yet unsufficient in underprivileged area of the world. This has been shown by several studies in the developing countries what reveals that Rotavirus is at 8% approximately responsible of diarrheal episodes with 28% for diarrhea consultation and 34% of children's hospitalisation between 0-59 months.

In 2009, the Democratic Republic of Congo through its broad program of vaccination launches an investigation with view to determine the incidence of this disease and the virulent strain in order to introduce a new vaccination against Rotavirus.

Methodology The current study targets children between 0-59 months after consulting Kingasani Hospitable Center from August 2009 to December 2017 for symptoms of acute diarrhea of at least 7 days coupled with vomiting and high temperature. The analysis of these data are done and confirmed by two significant tests: PCR and ELISA for genotyping.

Result 1628 cases of severe acute diarrhea have been registered in our Center since August 2009 to December 2017. All these stool samples have been swabbed and analysed by Rotavirus immunologic test of which 893 samples have been positive that to say 55%.

Conclusion For the prevention and protection of human life in general and particularly those of children between 0-59 months, exposed to the risk of Rotavirus affection, it has been decided to conduct the current investigation. It took place at Kingasani Hospitable Center. The fact is nearly 55% of children hospitalised in our Center have been victims of Rotavirus infection with acute diarrhea.

Their age vary between 0-59 months. Briefly, the above mentioned results demonstrate the necessity to introduce a vaccination against Rotavirus in the country with view to reduce the rate of children's mortality of at least 5 years in the Democratic Republic of Congo.

ABSTRACTS POSTERS

SECTION D: HOST AND MICROBE INTERACTIONS

Biosynthetic adaptations of *Brucella abortus* inside host cells

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Bacteria of the genus *Brucella* are collectively responsible for a worldwide zoonosis called brucellosis. These bacteria are mainly found inside host cells, colonizing several organs and massively replicating in the genital system.

The infection of host cells by *Brucella abortus* is roughly divided in two main steps. Soon after entry, bacteria follow the endosomal pathway and are retained for several hours in compartments having late endosomes markers. Thanks to a type IV secretion system, *B. abortus* is able to traffic to the endoplasmic reticulum (ER) where it proliferates. Bacterial growth in the ER was recently investigated using Tn-seq, a transpositional saturating mutagenesis allowing the identification of genes necessary for survival and growth in a given condition (Sternon et al., 2017). Tn-seq on *B. abortus* in RAW 264.7 macrophages revealed that only a few pathways (namely those for pyrimidines, histidine and branched-chain amino acids biosynthesis) seem to be required for growth in the ER. Even though the ER is thought to be a nutrient-rich compartment, it suggests that several key metabolites are in limited amount in the ER and therefore need to be synthesized *de novo* by *B. abortus*.

Deletion of selected enzymes from histidine and branched-chain amino acids biosynthesis pathways leads to auxotrophy for those amino acids in minimal medium. Besides, those deletion mutants are attenuated at 24h post-infection in HeLa cells and RAW 264.7 macrophages, confirming Tn-seq data.

The investigation of pathways that are crucial or dispensable for *B. abortus* allows a better description of the food sources available for *B. abortus* growth inside several host cell types.

The nature of lymphoid subpopulations associated to protective immunity against Brucella infection is strongly dependent of the route of infection

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Many infectious diseases are characterized by the persistence of the pathogen in their host. Chronicity and recurrence of these infections involve a long and costly therapy and cause significant morbidity. Vaccination constitutes the only prophylactic treatment that can reduce the societal cost of these infections.

Bacteria of the *Brucella* genus are facultatively extracellular intracellular Gram-negative coccobacilli that infect domestic and wild-type mammals but also humans. Human brucellosis is a debilitating and febrile chronic disease that constitutes a significant public health concern in areas of the world where *Brucella* infections are still endemic. Unfortunately, there is no available human brucellosis vaccine; all commercially available animal vaccines are live vaccines that can cause disease in humans.

Acquired immunity following infection relies primarily on the development of pathogen-specific memory lymphocyte populations. The first step in the rational selection of candidate vaccines is to identify lymphoid subpopulations associated with protective immunity. Here, we compared the course of *B. melitensis* infection in C57BL/6 mice infected via intraperitoneal (i.p.), intranasal (i.n.) and intradermal (i.d.) routes. We observed that the pattern of *Brucella* dissemination as well as the lymphoid subpopulations indispensable to control secondary infection appear dependent on the route of infection. For example, $CD4^+$ T cells are indispensable in i.p. model but dispensable for i.d. and i.n. models; B cells are indispensable in i.p. and i.d. models but dispensable for i.n. model. $\gamma\delta^+$T cells appear able to compensate the absence of $\alpha\beta^+$T cells in i.d. model but not in other models. Collectively, our results demonstrate that the nature of memory lymphoid subpopulations participating to the frontline immunity against *Brucella* infection varies according to the infection site. This observation suggests that a vaccine must induce the development of lymphoid subpopulations associated to all natural routes of infection to be fully protective.

Contribution of the β-glucosidase BglC to the onset of the pathogenic lifestyle of *Streptomyces scabies*

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Common scab disease on root and tuber plants is caused by *Streptomyces scabies* and the few related pathogenic Streptomycetes which use the cellulose synthase inhibitor thaxtomin A as the main phytotoxin. Thaxtomin production is primarily triggered by the import of cellulose oligosaccharides (cello-oligosaccharides) cellobiose and cellotriose. Once inside the cell, the fate of the cello-oligosaccharides is dichotomized: (i) the fuelling of glycolysis with glucose for the saprophytic lifestyle through the action of β-glucosidase(s) (BGs); and (ii) elicitation of the pathogenic lifestyle by the inhibition of CebR-mediated transcriptional repression of thaxtomin biosynthetic genes. Here, we investigated the role of *scab57721*, encoding a novel BG (BglC), in the onset of the pathogenicity of *S. scabies*. Enzymatic assays conducted on the purified BglC enzyme showed that it releases glucose from cellobiose, cellotriose and all other cello-oligosaccharides tested. Its inactivation resulted in a mutant phenotype opposite to that expected, as reduced production of thaxtomin was monitored when the mutant was cultivated on minimal medium containing cello-oligosaccharides as unique carbon source. This unexpected phenotype could be attributed to a surprisingly increased activity of alternative intracellular BGs, probably as a compensation for *bglC* inactivation, which then prevented cellobiose and cellotriose accumulation to reduce the activity of CebR. In contrast, when the *bglC* null mutant was cultivated on medium devoid of cello-oligosaccharides, it instead constitutively produced thaxtomin. Additionally, this mutant strain also overproduced thaxtomin compared to the wild-type strain in cellobiose-containing media. These differences in thaxtomin production resulted in a hypervirulent phenotype but do not fit with the proposed model of the cello-oligosaccharide-mediated induction of thaxtomin production. The role of BglC in the route to the pathogenic lifestyle of *S. scabies* is most likely more complex than currently presented.

Host-microbe interaction in the upper respiratory tract: development of a polymicrobial model of the sinonasal microenvironment

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Mechanistic research to unravel the aetiology and pathology of chronic rhinosinusitis and to find innovative treatment methods requires models of the affected tissues and their resident microbiota. A co-culture model of the upper respiratory tract with a representative epithelial structure, immune cells and representative microbial communities from healthy individuals was established. THP-1 derived macrophages were included in the co-culture model at the basal side of the epithelial cell layer, using an extracellular matrix coating. In order to examine the effect of different inflammatory conditions on both epithelial cell layers and the microbial community, non-activated (M0), classically activated (M1) and alternatively activated (M2) THP-1 derived macrophages were incorporated in the set-up and a control group without macrophages was included. Nasal microbiota derived from two healthy donors were used to inoculate the different human cell cultures and the triple co-cultures were maintained for 48 hours. Host cell cytotoxicity was assessed by lactate dehydrogenase release, tight junction functionality was measured as transepithelial electrical resistance, and IL-8 release was measured using ELISA. Microbial community profiling was performed using phenotypic fingerprinting from flow cytometry data. Cytotoxicity remained below 10 % for all experimental groups and did not differ significantly between different human cell cultures or between donors. Epithelial resistance was maintained in cell layers co-cultured with classically activated macrophages, whereas it decreased in co-cultures without macrophages and with M0 macrophages. In contrast, in co-cultures with M2 macrophages, an increase in epithelial resistance was observed. Presence of donor-derived microbiota did not impact epithelial resistance. This polymicrobial, immunocompetent model could enable steering of the inflammatory state to investigate host-microbe interactions in healthy and inflammatory upper respiratory tract conditions. The use of individual-derived samples can help to personalize the *in vitro* model and better predict the response to therapeutics, considering the microbiome as key factor in modulating the inflammatory response in the sinonasal environment.

The role of streptodornases in Group A *Streptococcus* virulence.

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Group A *Streptococcus (*GAS) is a major cause of morbidity globally and causes more than 500.000 deaths per year. GAS engenders a broad spectrum of diseases ranging from uncomplicated pharyngitis and skin infections to life-threatening invasive illnesses as well as non-suppurative immune-mediated sequelae, acute rheumatic fever and glomerulonephritis. GAS virulence is due to a complex interplay between host factors and secreted or surface-bound bacterial factors, including proteases, phospholipases, DNases and superantigens.

Our principal objective is to investigate the contribution of DNases to virulence and pathogenesis of invasive GAS disease using an isolate recovered from a particularly severe clinical presentation and called L01. Previous studies support the importance of DNases in GAS virulence and in the switch between localized and systemic infections. As the L01 has 4 DNases (Sdn, Spd1, Spd3 and Spd4), we want to investigate their individual role in the L01 pathology, as well as their potential cumulative effect on virulence.

For this purpose, we have cloned and produced 3 of these DNases (Sdn, Spd1, Spd3) as recombinant proteins. We have shown that the 3 DNases are able to degrade eukaryotic and prokaryotic, genomic and plasmidic DNA and that mutation of the conserved catalytic residue of each DNase totally abolishes their enzymatic activity. We have shown that the 3 DNases are active at different temperatures (ranging from 28 to 37°C) and also at the low pH encountered in necrosis and phagolysosomes. Despite their enzymatic activity, our preliminary data shown that the 3 DNases are unable to degrade NETs.

In parallel, we have monitored the expression of their genes by RT-qPCR in different conditions. We have shown that the 4 DNases, even if all expressed in the exponential growth phase, are much more expressed during the stationary growth phase (between 4 and 30 times more). Their expression is slightly increased after serum passage, except for Sdn whose expression is 10 times lower than in the exponential phase.

Finally, we have also shown that the L01 isolate has the capacity to form biofilms and that the DNases are expressed in the biofilm nearly at the same level than in the stationary phase. Our preliminary data have shown that the DNases were able to disperse a biofilm from *Pseudomonas aeruginosa*.

Identification of bacterial genes indispensable to the early phase of pulmonary infection by *Brucella melitensis* in mice

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Infection by the bacteria of the genus *Brucella* called brucellosis are common worldwide for both mammals and humans. In the actual context with still no efficient and save vaccine available, brucellosis still represents huge economical losses for endemic countries. Causing notably sterility and abortion, containment of the disease rely on mass slaughter of the cattle. Moreover chronicity and recurrence of this infection cause significant morbidity in human despite long and costly antibiotic treatments. The aim of this study is to decipher how this pathogenic bacteria adapt to the specific microenvironment of the host and successfully evade the first defense lines of the immune system before resulting in chronic infection.

To obtain a timeline of the *Brucella* infectious cycle in the mice lungs after intranasal infection, we used an *in situ* visualization approach of the bacterial multiplication with a fluorescent reporter system. Our observations in fluorescence microscopy suggest that replicative bacteria are more sensitive to the immune response. A fraction of the non-growing bacteria that survive the first 24h, later give rise to an intensive proliferation in some alveolar macrophages. The outcome of the infection at the cell level can be influenced to some extend by immune deficiencies. This replication control may reflect a strategy to bypass the immunity and have time to adapt to the cell microenvironment.

Using this timeline, our goal was to identify which are the genes that are required for the colonization of mice lungs and since what time they are at play. To do so, we used a high density transposon insertion site sequencing (Tn-Seq) approach on the whole genome of *Brucella melitensis*. Using this method at selected time point during the first 72 hours of *Brucella* pulmonary infection, our analysis highlighted 116 candidates that contribute to efficient host colonization by *Brucella.* Some of these candidates are linked to virulence gene expression but most of them can be associated with a deep adaption to microenvironment of the host cell.

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Using interactions between *Hermetia illucens* and microbes to increase the microbial safety and growth performance of the larvae - a literature evaluation.

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The Black Soldier Fly (BSF, *Hermetia illucens*) has become one of the most important insects in the world for bioconversion, as its larvae are able to valorise low quality organic waste streams (e.g. household organic waste). Such waste streams often have a high microbial load, raising questions on the microbial safety of the larvae reared on these substrates. We performed an extensive literature search[1] to explore how these larvae could cope with the presence of various micro-organisms and the impact their presence will have on the growth and microbial content of the larvae. Our search points at the existence of a set of currently hardly explored mechanisms in place that could avoid the microbial community in the BSF larvae from being taken-over by specific bacteria on a range of substrates, potentially in different stages of decay. This is evidenced by the observation that the counts of two pathogens, *Escherichia coli* O157:H7 and *Salmonella* spp., were found to be reduced in the substrate in the presence of the larvae[2]. However, the underlying mechanisms are not yet identified. At the same time, larval immunity is found to react to the presence of micro-organisms with the production of antimicrobial peptides that could kill these micro-organisms[3]. An overview of these findings will be presented, combined with the needs for future research to explore to what extent food pathogens, potentially present in the substrate, can colonize the BSF microbiome, and if and how modulation of BSF immunity will impact colonization. This research will lead to novel strategies to exploit the numerous host-microbe interactions that occur in *Hermetia illucens*.

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Interactions between *Propionibacterium acnes* and different human cell types are strain-dependent

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Propionibacterium acnes is one of the most prevalent members of the human skin flora. Colonization of the pilosebaceous units by this bacterium is considered an important contributing factor in the pathogenesis of acne. Recent studies have shown that *P. acnes* populations differ considerably between acne patients and healthy individuals, with some strains being more prevalent in normal skin while others appear to be associated with acne.

The goal of the present study is to determine whether differential association of *P. acnes* strains with acne or healthy individuals is due to differential interactions with relevant human skin cells (SZ95 sebocytes and HaCaT keratinocytes).

Adhesion of various *P. acnes* strains (associated with healthy skin, acne or both) to SZ95 and HaCaT cells was determined after 24 h and 48 h of coculture followed by plating through microdilution. The release of the proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α of both skin cell lines during coculture was quantified using ELISA assays. In addition, the influence of the different *P. acnes* strains on sebaceous lipid synthesis was investigated using Oil red O as a lipid detection dye. For all experiments a multiplicity of infection of 10:1 was used.

Plating of *P. acnes* showed an overall higher adhesion of acne-associated strains to SZ95 sebocytes after 48 h of coculture, compared with healthy-associated strains. This difference was not detected in HaCaT cells. Preliminary results show that all strains tested were able to induce IL-6 and IL-8 release in both SZ95 sebocytes and HaCaT keratinocytes, but no significant differences were observed between the tested strains. Interestingly, acne-associated *P. acnes* strains induced higher levels of IL-1β and TNF-α in SZ95 sebocytes than *P. acnes* strains associated with healthy skin. A similar trend for TNF-α was found in HaCaT keratinocytes, although not statistically significant. Lipid production by SZ95 sebocytes was not affected by the presence of *P. acnes*.

Our results demonstrate that *P. acnes* strains typically associated with acneic or healthy skin interact differently with human skin cells, although a wider panel of *P. acnes* strains needs to be tested to confirm these differences.

The stress-related alarmone (p)ppGpp regulates the cell cycle of *Brucella abortus* and its virulence

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Brucella spp. are facultative intracellular bacteria responsible for Brucellosis, a worldwide anthropozoonosis. This neglected disease is found in a variety of mammals and humans are considered as accidental hosts. The genome of *B. abortus* is divided into two chromosomes named chromosome I (ChrI) and chromosome II (ChrII) with a size of 2.1 Mb and 1.2 Mb respectively (Moreno *and* Moryon, 2006).

Recently several tools were developed to monitor the state of the chromosomes replication throughout the cell cycle of *B. abortus* at the single cell level. Indeed, by highlighting proteins involved in the partitioning system of chromosomal replication origins (*oriI* and *oriII*), we are able to determine the number of replication origins of both chromosomes indicating if the bacterium is in G1 phase (no DNA replication) or in S/G2 phase (DNA replication is occurring or is finished) (Deghelt *et al*., 2014).

Using these reporter strains to infect RAW 264.7 macrophages and epithelial HeLa cells, we showed that *B. abortus* presents a biphasic infection process. Indeed, the infection is characterized by a first non-proliferative step where bacteria are arrested in G1 phase, the invasion form of *B. abortus* in these host cells, followed by a proliferative step where bacteria grow and replicate in the endoplasmic reticulum (Deghelt *et al*., 2014). Since the cell cycle of *B. abortus* seems to be directly linked to its infection strategy, we are interested to investigate the regulation of this cell cycle and more precisely the control of the chromosomes replication in rich medium and during infection.

Previous work highlighted the role of the alarmone (p)ppGpp in the response to environmental changes and more particularly to nutritional stresses. In bacteria, the synthesis and degradation of this alarmone are controlled by RelA/SpoT protein family. In *Escherichia coli* RelA produces (p)ppGpp from GTP and ATP while SpoT can both synthesize and hydrolize (p)ppGpp. Some other bacteria (including *C. crescentus* and *B. abortus*) have only one protein having both synthetase and hydrolase activities, these proteins are named RSH for RelA SpoT Homologues. Overproduction of (p)ppGpp in the alphaproteobacteria *Caulobacter crescentus* leads to a growth defect and a G1 arrest (Lesley and Shapiro 2008). A Δ*rsh* mutant of *Brucella suis* is unable to proliferate in THP-1 macrophages, suggesting that (p)ppGpp could play a major role in the adaptation of *Brucella* to its intracellular niche (Dozot *et al*., 2006).

In order to study more deeply the role of the alarmone in *B. abortus* we overexpressed the *relA* gene from *E. coli* in *B. abortus* via an inducible promoter, which resulted in a growth defect in rich culture medium as well as a decrease in bacteria sizes compared to the wild-type and the non-induced strain. Moreover, flow cytometry analysis of the *relA*-expressing *B. abortus* strain shows an increase of the proportion of G1 bacteria in rich culture medium suggesting a link between (p)ppGpp production and an inhibition of DNA replication initiation. This result is confirmed by the generation of a strain overexpressing *relA* where the replication of chromosome I is monitored. Indeed, in the non-induced *relA* strain the G1 bacteria represent 20% of the population whereas the induction of *relA* for six hours leads to an increase up to 35%. To confirm the involvement of (p)ppGpp in all these phenotypes a negative control was generated consisting in the production of *relA** that contains a point mutation leading to an inactive (p)ppGpp synthetase domain. The resulting strain does not show any growth delay nor G1 bacteria accumulation. In addition, the induction of *relA* during infection of RAW 264.7 macrophages and HeLa cells abrogates bacterial proliferation inside host cells. Altogether these data suggest that production of (p)ppGpp has an impact on the cell cycle progression as well as on the ability of *B. abortus* to establish a successful infection.

Influence of bacteria from the COPD lung microbiome on cigarette smoke-induced pro-inflammatory responses of three-dimensional lung epithelial cells

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Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide and is characterized by an excessive inflammatory response of the lung to harmful substances. Although the pathogenesis of COPD is still under investigation, smoking is the most important risk factor for developing COPD. It has been reported that microbial composition of the lungs of patients with COPD is different from that of healthy individuals and differs depending on the disease state. However, limited research has been done to understand if the lung microbiota contribute to the onset of cigarette smoke-induced COPD and disease progression. In order to explore this question, bacteria that are frequently isolated from the COPD microbiome were evaluated for their pro-inflammatory potential, including *Pseudomonas aeruginosa, Acinetobacter baumannii, Rothia mucilaginosa, Haemophilus influenzae, Moraxella catarrhalis, Neisseria meningitidis* and *Veillonella parvula*. To this end, a three-dimensional (3-D) in vivo-like tissue culture model (A549 cell line) which reflects morphological and functional features of lung epithelium was exposed to cigarette smoke extract (CSE) in the presence and absence of the seven microbiome members (MOI 10, 4h) and the production of the pro-inflammatory cytokine IL-8 was measured. Results point out that in the absence of CSE differences in IL-8 production occur depending on the species tested, some species being strong inducers of IL-8 while others weakly induced IL-8. In addition, we observed that *R. mucilaginosa* completely abolished the CSE-induced IL-8 production. We also confirmed these findings in bronchial epithelial cells (16HBE cell line). In conclusion, certain members of the COPD microbiome elicit pro-inflammatory responses in lung epithelial cells, while others affect the CSE-induced IL-8 production. These initial findings suggest a contribution of the COPD microbiome to the overall inflammatory profile in the COPD lung environment..

Interactome analysis of the non-structural proteins 1 and 2 of human respiratory syncytial virus

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Human respiratory syncytial virus (HRSV) is a common respiratory pathogen. HRSV encodes two non-structural (NS) proteins 1 and 2, that strongly counteract the type I and III IFN mediated host antiviral response. NS1 and -2 independently and cooperatively suppress both type I IFN production and - signaling at multiple stages.

To better understand the functions of HRSV-NS1 and –NS2 in counteracting the antiviral host responses, we investigated the NS1 and NS2 protein-protein interactome by using a proximity based biotin ligation assay, called BioID. In this technique, we fused NS1 and NS2 genetically to a point mutant (BirA*) of the *Escherichia coli* biotin ligase BirA that makes this enzyme promiscuous in terms of its substrate specificity. This fusion protein is overexpressed in the presence of an excess of free biotin. As a result, proteins in the proximity of the bait-BirA* protein are biotinylated. Upon cell lysis biotinylated proteins can be enriched using streptavidin and identified by mass spectrometry.

We performed this BioID screen twice. During the first screen, 836 and 345 proteins were identified as the NS1 and NS2 proxeome respectively, whereas in the second screen respectively 426 and 146 proteins were significantly enriched upon NS1- and NS2-BirA* overexpression. As control we also investigated the proxeome of an irrelevant protein, the human kinase MYLK4.
When the first and the second BioID screen were compared for overlapping proteins, 64.5 % of the proteins identified in the NS1 proxeome were common. For the NS2 proxeome, the percentage of overlapping proteins was 69.9%

When the proxeome data were analysed by IPA, the Cleavage and polyadenylation of premRNA pathway was identified as highly enriched in the NS1 proxeome. Upon interaction with these host proteins, host gene expression can be regulated, possibly to favor viral replication.

Currently, we are focusing on the NS1 and NS2 proxeome in a more natural context of RSV infection. We plan to generate recombinant RSV viruses in which we fuse the BirA* protein to NS1, NS2 or the small hydrophobic protein as a control by use of homologous recombination. For recombinant virus recovery, we will use a reverse genetics system based on a BAC clone that encodes the entire RSV antigenome under control of a T7 promoter. When this BAC clone, together with the helper plasmids (N,L,P,M2-1) is co-transfected into BSR-T7/5 cells, recombinant RSV virus can be produced. By applying this system with endogenous NS1- and NS2 BirA* expression, hopefully we can eliminate artefacts characteristic to overexpression of proteins of interest.

Our ultimate goal is to investigate NS1 and NS2 interaction partners, e.g. also in cells that are derived from human respiratory epithelium such as the A549 cell line and to elucidate the role in the RSV life cycle of newly identified NS1 and NS2 interaction partners.

Steering the tongue microbiome through a nitrate rich diet

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The oral microbiome is one of the most diverse microbial communities in the human body. Moreover its members can perform a wide range of metabolic processes and they can influence oral and even systemic health. One example is the oral nitrate reducing bacteria that convert nitrate to nitrite performing the first step of the entero-salivary nitric oxide pathway. These play an important role in the NO homeostasis of the human body, which is positively correlated with cardiovascular function. On the other hand, the tongue dorsum harbours anaerobic bacteria that produce volatile sulfur compounds (mainly H_2S), leading to halitosis (oral malodour). Sulfate and nitrate reducing bacteria are known to act antagonistically in other environments, as they compete for the same electron donors.

In this study we investigated if an increase in nitrate can stimulate denitrifying bacteria to counter sulfate reducing bacteria, in order to steer the tongue microbiome activity from H_2S production to the more favourable nitrate reduction. We conducted an intervention study where a group of individuals followed a nitrate rich diet for a week by daily consumption of beetroot juice. A second group remained under regular diet habits and acted as a control group. The tongue dorsum of the individuals was sampled pre- and post-treatment. 16S rRNA gene amplicon sequencing was used to study the tongue microbiome composition and the potential shifts due to the increased nitrate consumption. We identified a core baseline microbiome of 40 OTUs which changed upon treatment. We observed an increase in alpha diversity after the nitrate rich diet, yet without any OUT-related shifts. We thus demonstrate that a short diet intervention can indeed have an impact on the composition of the tongue microbiome. Nevertheless a next step with inclusion of halitosis positive individuals and addition of metabolic data is important in order to define if nitrate rich diet can act as a non-invasive treatment for halitosis.